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MODIFICATION OF CYTOCHROME P-450, NADPH-CYTOCHROME *c* REDUCTASE AND ARYL HYDROCARBON HYDROXYLASE ACTIVITIES BY SCHISTOSOMICIDAL DRUGS

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Abstract—This study was planned to investigate the modification of the mouse microsomal monooxygenase enzymes using various schistosomicidal drugs. Enzymes investigated were cytochrome P-450, NADPH-cytochrome *c* reductase and aryl hydrocarbon hydroxylase (AHH). Administration of oxamniquine and niridazole increased, whereas praziquantel and hycanthone lowered the cytochrome P-450 content. An apparent increase in the activity of NADPH-cytochrome *c* reductase was only observed with oxamniquine. The *in vivo* and *in vitro* effects of schistosomicidal drugs on the activity of AHH were investigated using benzo(*a*)pyrene (BP) as substrate. Oxamniquine and niridazole significantly increased the AHH activity *in vivo* and *in vitro*, while the antimonial drugs enhanced the enzyme activity only *in vivo*. On the other hand, praziquantel and hycanthone lowered the AHH activity only *in vivo*. Metrifonate did not show any effect either *in vivo* or *in vitro*. The mechanisms by which these drugs modify the AHH activity are discussed in the text.

Various xenobiotics have been reported to alter the activity of the drug-metabolizing enzyme systems, mainly the membrane-bound mixed-function oxygenases, which have as their terminal electron acceptor the cytochrome P-450; they are both metabolized and induced by a wide spectrum of exogenous compounds including drugs, pesticides, polycyclic hydrocarbons, food additives and endogenous substrates such as steroids. Any alterations in these enzyme activities may affect the intensity and duration of drug action as well as the rate at which these various compounds are metabolized, to yield either inert or toxicologically active metabolites [1–3].

Because of the increasingly widespread use of schistosomicidal drugs in Egypt, especially among agricultural societies in rural areas where schistosomiasis is endemic, considerable concern exists regarding their toxicological and/or carcinogenic hazards since some of them were reported to be directly mutagenic and carcinogenic [4, 5].

However, these drugs might also alter the carcinogenicity of some chemical carcinogens including benzo(*a*)pyrene by modifying the metabolizing enzymes responsible for their biotransformation to reactive metabolites.

This study was undertaken in an attempt to shed some light on the effect of various commonly used schistosomicidal drugs on the microsomal cytochrome P-450, NADPH-cytochrome *c* reductase and the benzo(*a*)pyrene hydroxylase activities.

MATERIALS AND METHODS

Animals. Six-week-old male Swiss albino mice weighing 18–25 g were obtained from the animal house, High Institute of Public Health, Alexandria University, Alexandria, Egypt. They were kept in cages, five to a cage. All animals were maintained on a standard laboratory diet and were given food and water *ad libitum*, except for 24 hr before decapitation.

Administration schedules. Each schistosomicidal drug was administered to two different groups of mice, the first group received a single dose of the drug whereas the other group received the same dose every 24 hr for three consecutive days. Anthiomaline (Alex. Co. for Drugs and Chemical Industries, Alexandria, Egypt) was administered i.p. in distilled water at a dose of 10 mg/kg body weight. Astiban (Roche and Co. Ltd., Basle, Switzerland) was administered i.p. in distilled water at a dose of 40 mg/kg body weight. Oxamniquine (Pfizer Egypt, SAA Cairo, Egypt) was adminstered orally in corn oil at doses of 40 mg and 200 mg/kg body weight. Praziquantel (gift from Prof. Somasak Ruchirawat, Mahidol University, Bangkok, Thailand) was administered orally in corn oil at a dose of 60 mg/kg body weight. Niridazole (Ciba Geigy Ltd., Basle, Switzerland) was administered orally in corn oil at a dose of 250 mg/kg body weight. Metrifonate (Bayer Leverkusen, F.R.G.) was administered orally in corn oil at a dose of 100 mg/kg body weight. Hycanthone (Winthrop Products Inc. New York, U.S.A.) was administered i.p. in distilled water at a dose of 30 mg/kg body weight. The corresponding controls for each treatment received an equal volume of the vehicle.

Enzyme determinations. The animals were killed 24 hr after the last injection. Livers were homogenized in 3 vol. (w/v) of 0.1 M potassium phosphate buffer pH 7.4, and centrifuged for 20 min at 11,000 *g*. The supernatant fraction was centrifuged

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Table 1. Effect of single dose treatment of some schistosomicidal drugs on the hepatic cytochrome P-450 content, the NADPH-cytochrome *c* reduyctase and the aryl hydrocarbon hydroxylase activities

Treatment	Cytochrome P-450 content (nmoles/mg microsomal protein)*		NADPH-cytochrome *c* reductase activity (nmoles/mg microsomal protein/min)*		Aryl hydrocarbon hydroxylase activity (pmoles 3-OH-BP/mg microsomal protein/min)*	
	Control†	Treated	Control†	Treated	Control†	Treated
Anthiomaline (10 mg/kg body wt., i.p. in distilled water)	1.263 ± 0.047	1.435 ± 0.086 No effect NS‡	86.91 ± 9.42	84.63 ± 9.35 No effect NS‡	101.4 ± 9.30	111.6 ± 5.01 No effect NS‡
Astiban (40 mg/kg body wt., i.p. in distilled water)	1.112 ± 0.062	1.001 ± 0.093 No effect NS‡	57.76 ± 4.55	55.82 ± 3.29 No effect NS‡	58.4 ± 8.01	56.4 ± 5.72 No effect NS‡
Oxamniquine (40 mg/kg body wt., orally in corn oil)	1.055 ± 0.084	1.074 ± 0.100 No effect NS‡	57.65 ± 3.35	63.04 ± 4.10 No effect NS‡	78.2 ± 1.97	124.8 ± 12.20 60% increase $P < 0.01$
Oxamniquine (200 mg/kg body wt., orally in corn oil)	0.862 ± 0.078	1.032 ± 0.092 No effect NS‡	62.88 ± 2.24	73.13 ± 4.38 No effect NS‡	64.4 ± 10.77	108.0 ± 11.43 68% increase $P < 0.05$
Praziquantel (60 mg/kg body wt., orally in corn oil)	1.195 ± 0.104	0.723 ± 0.092 40% decrease $P < 0.01$	65.25 ± 4.34	62.46 ± 3.96 No effect NS‡	97.6 ± 16.67	48.6 ± 4.83 50% decrease $P < 0.05$
Niridazole (250 mg/kg body wt., orally in corn oil)	0.991 ± 0.105	1.129 ± 0.050 No effect NS‡	65.74 ± 5.52	81.40 ± 5.32 No effect NS‡	79.4 ± 9.67	84.2 ± 6.39 No effect NS‡
Metrifonate (100 mg/kg body wt., orally in corn oil)	0.889 ± 0.047	0.846 ± 0.064 No effect NS‡	64.74 ± 4.84	59.29 ± 3.68 No effect NS‡	78.2 ± 8.77	89.8 ± 4.70 No effect NS‡
Hycanthone (30 mg/kg body wt., i.p. in distilled water)	1.233 ± 0.113	0.848 ± 0.103 31% decrease $P < 0.05$	64.50 ± 5.42	57.31 ± 6.27 No effect NS‡	52.8 ± 4.59	33.4 ± 1.35 37% decrease $P < 0.01$

* Values are the mean ± SE of five mice.
† Control mice received an equivalent volume of the vehicle and were assayed together with the treated mice.
‡ Value is not significant statistically.

at 105,000 *g* for 1 hr and the microsomal pellet was resuspended in 0.1 M phosphate buffer. Cytochrome P-450 was determined by the method of Omura and Sato [6], using 91 mM^{-1} cm^{-1} as the molar extinction coefficient for the reduced cytochrome P-450–CO complex. NADPH-cytochrome *c* reductase was assayed by following the reduction of cytochrome *c* at 550 nm using an extinction coefficient of 21 mM^{-1} according to Williams and Kamin [7]. The determination of AHH activity was performed by the method of Wiebel and Gelboin [8]. In the *in vitro* assay of AHH, schistosomicidal drugs dissolved in 0.1 M potassium phosphate buffer, pH 7.4 were added to the incubation mixture. Drugs showing significant effects on the enzyme activity were then prepared as different serial millimolar concentrations ranging from 10^{-8} M to 10^{-2} M. In all these experiments the same volume of 0.1 M potassium phosphate buffer, pH 7.4, was added to the incubation medium of the control. The protein was determined by the method of Lowry *et al.* [9]. Student's *t*-test was performed on the data and probability values (P) of less than 0.05 were considered significant.

RESULTS

The effects of single dose treatment of various schistosomicidal drugs on the microsomal enzyme activities are shown in Table 1. None of the tested drugs altered the microsomal protein or the liver:body weight ratio (unpublished data) or the NADPH-cytochrome *c* reductase activity. However, marked depression of the cytochrome P-450 content and AHH activity with either praziquantel or hycanthone pretreatments was shown. In addition, the AHH activity was increased when mice were treated with oxamniquine at two dose levels.

The effects of repeated dose treatment are shown in Table 2. None of the tested drugs altered the liver:body weight ratio. Oxamniquine (in the applied dose) and niridazole significantly increased the cytochrome P-450 content. On the other hand, hycanthone markedly lowered the cytochrome P-450 content. The depression was more pronounced than that encountered during single dose treatment. From all the tested drugs only oxamniquine enhanced the NADPH-cytochrome *c* reductase activity. It was also found that oxamniquine, niridazole, anthiomaline and astiban pretreatments significantly enhanced the AHH activity, while praziquantel and hycanthone pretreatments greatly inhibited the activity of the enzyme.

As can be seen from Table 3, only oxamniquine and niridazole increased the activity of AHH *in vitro*. The effect of increasing concentrations of either drug was studied using different concentrations ranging

Table 2. Effect of repeated dose treatment of some schistosomicidal drugs on the hepatic cytochrome P-450 content, the NADPH-cytochrome *c* reductase and the aryl hydrocarbon hydroxylase activities

Treatment	Cytochrome P-450 content (nmoles/mg microsomal protein)†		NADPH-cytochrome *c* reductase activity (nmoles/mg microsomal protein/min)†		Aryl hydrocarbon hydroxylase activity (pmoles 3-OH-BP/mg microsomal protein/min)†	
	Control‡	Treated	Control‡	Treated	Control‡	Treated
Anthiomaline (10 mg/kg body wt., i.p. in distilled water)	1.263 ± 0.047	1.291 ± 0.058 No effect NS§	86.91 ± 9.42	81.97 ± 5.92 No effect NS§	101.4 ± 9.30	133.0 ± 9.13 31% increase $P < 0.05$
Astiban (40 mg/kg body wt., i.p. in distilled water)	1.112 ± 0.062	1.260 ± 0.108 No effect NS§	57.76 ± 4.55	62.58 ± 2.29 No effect NS§	58.4 ± 7.00	79.2 ± 4.28 36% increase $P < 0.05$
Oxamniquine (40 mg/kg body wt., orally in corn oil)	1.055 ± 0.084	1.462 ± 0.071 39% increase $P < 0.01$	57.65 ± 3.35	73.38 ± 5.10 27% increase $P < 0.05$	78.2 ± 1.97	131.6 ± 7.84 68% increase $P < 0.001$
Oxamniquine (200 mg/kg body wt., orally in corn oil)	0.862 ± 0.078	1.356 ± 0.040 57% increase $P < 0.001$	62.88 ± 2.24	81.18 ± 3.71 29% increase $P < 0.01$	64.4 ± 10.77	128.0 ± 14.20 99% increase $P < 0.01$
Praziquantel (60 mg/kg body wt., orally in corn oil)	1.195 ± 0.104	0.965 ± 0.068 No effect NS§	65.25 ± 4.34	61.32 ± 2.40 No effect NS§	97.6 ± 16.67	53.0 ± 8.90 46% decrease $P < 0.05$
Niridazole (250 mg/kg body wt., orally in corn oil)	0.991 ± 0.105	1.444 ± 0.088 46% increase $P < 0.05$	65.74 ± 5.52	76.68 ± 3.74 No effect NS§	79.4 ± 9.67	117.6 ± 8.77 48% increase $P < 0.05$
Metrifonate (100 mg/kg body wt., orally in corn oil)	0.889 ± 0.047	0.960 ± 0.064 No effect NS§	64.74 ± 5.52	59.29 ± 3.68 No effect NS§	78.2 ± 8.77	82.2 ± 6.25 No effect NS§
Hycanthone (30 mg/kg body wt., i.p. in distilled water)	1.233 ± 0.113	0.723 ± 0.068 41% decrease $P < 0.01$	64.50 ± 5.42	51.92 ± 5.32 No effect NS§	52.8 ± 4.59	17.8 ± 0.91 66% decrease $P < 0.001$

* Drugs were administered to mice for three consecutive days.
† Values are the mean ± SE of five mice.
‡ Control mice received an equivalent volume of the vehicle and assayed together with the treated mice.
§ Value is not significant statistically.

Table 3. *In vitro* effect of various schistosomicidal drugs on mouse liver microsomal aryl hydrocarbon hydroxylase activity

Treatment*	pmoles 3-OH-BP/mg microsomal protein/min†	% Change
Control	80.0 ± 1.29	—
Anthiomaline	85.5 ± 2.44 (NS‡)	No effect
Astiban	82.4 ± 4.36 (NS‡)	No effect
Oxamniquine	189.7 ± 5.08 ($P < 0.001$)	137% increase
Praziquantel	87.6 ± 3.48 (NS‡)	No effect
Niridazole	122.8 ± 4.03 ($P < 0.001$)	54% increase
Metrifonate	82.1 ± 2.87 (NS‡)	No effect
Hycanthone	73.1 ± 2.81 (NS‡)	No effect

* All drugs were added to the microsomal incubation medium as 10^{-4} M concentration in 0.1 M potassium phosphate buffer.
† Values are the mean ± SE of four experiments.
‡ Value is not significant statistically.
§ The same volume of the phosphate buffer was added to the incubation medium of the controls.

from 10^{-8} M to 10^{-2} M (Figs 1 and 2). Oxamniquine enhances the activity of AHH upon increasing the drug concentration, reaching a maximum at 10^{-4} M followed by a gradual decrease and finally returning to normal. However, niridazole produced a maximum activity at 5×10^{-8} M.

DISCUSSION

The main purpose of this study is to investigate the ability of schistosomicidal drugs to modify the activities of some hepatic oxidative drug-metabolizing enzymes.

Repeated dose treatment of either oxamniquine

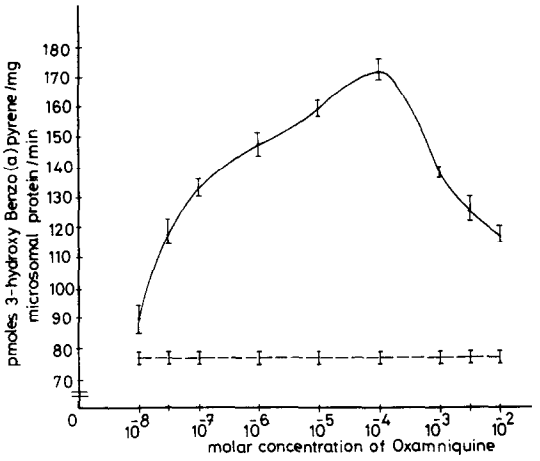

Fig. 1. Increase of aryl hydrocarbon hydroxylase activity of the mouse liver microsomes with different concentrations of oxamniquine *in vitro*. The assays are carried out as described in Materials and Methods: -----, assay without oxamniquine; ——, assay with different concentrations of oxamniquine. Values are expressed as the mean ± SE for four determinations..

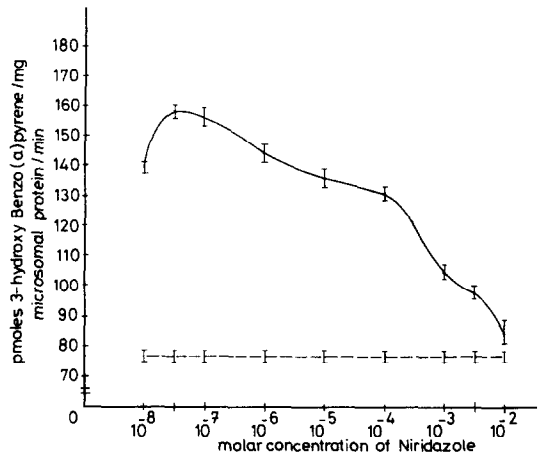

Fig. 2. Increase of aryl hydrocarbon hydroxylase activity of mouse liver microsomes with different concentrations of niridazole *in vitro*. The assays are carried out as described in Materials and Methods: -----, assay without niridazole; ——, assay with different concentrations of niridazole. Values are expressed as the mean ± SE for four determinations.

(quinoline derivative) and niridazole (imidazole–thiazole derivative) caused a marked increase in the cytochrome P-450 content. This effect is in agreement to that observed during pretreatment of rabbits with imidazole which resulted in a two-fold increase in the hepatic microsomal cytochrome P-450 content [10, 11]. The effect of praziquantel and hycanthone on the hepatic cytochrome P-450 was different from that of the above mentioned drugs, since single dose treatment of mice markedly decreased the amount of cytochrome P-450. This apparent decrease may result from the denaturation of the cytochrome P-450 heme moiety, as observed with most inhibitors of mixed function oxidases [12].

Concerning the NADPH-cytochrome *c* reductase, only oxamniquine produced a significant increase in the activity of the enzyme upon repeated treatment with both applied dose levels. The activation of the reductase may result in the enhancement of the rate of reduction of cytochrome P-450 [13].

The *in vivo* assay of AHH showed that oxamniquine increased the enzyme activity when administered both as single and repeated doses. On the other hand, niridazole and the antimonial drugs, anthiomaline and astiban, increased the activity when administered as a repeated dose alone. Contrary to this effect, praziquantel and hycanthone decreased the activity when administered both as single and repeated doses (Tables 1 and 2). Therefore, it seems that the magnitude of alteration of AHH activity differs according to the type of the drug tested and to the duration time of the drug administration. However, many other factors have also been reported to alter the activity of AHH although the mechanisms of alteration might differ from one compound to another according to the chemical structure and the different groups substituted in the compound [14]. It is well documented that AHH is a cytochrome P-450 dependent enzyme [15–17] and the data obtained from this study provide further evidence for this dependency since most of the tested drugs which either increased or decreased the activity of AHH had similar effects on the cytochrome P-450 content (Tables 1 and 2).

In vitro study showed that from all the schistosomicidal drugs only oxamniquine and niridazole significantly altered the AHH activity. Oxamniquine yielded a nearly bell-shaped curve with maximum activity at 10^{-4} M, while niridazole resulted in a descending stepwise curve having maximum activity at 5×10^{-8} M (Figs 1 and 2). Since the *in vitro* effects of both drugs on the AHH activity resemble those observed *in vivo*, it may be suggested that the *in vivo* and *in vitro* inductions by these drugs are regulated by a similar mechanism, which could be attributed to the drugs themselves rather than their metabolites. In case of praziquantel and hycanthone, the inhibitory effect shown *in vivo* was not seen in the *in vitro* experiment, thus suggesting their requirement for prior metabolism in order to exert their effects. The last drug, metrifonate, did not alter the activities of any of the investigated enzymes.

The results of this study demonstrate the ability of various schistosomicidal drugs to alter the activities of drug-metabolizing enzyme and since some of these drugs are themselves mutagenic and carcinogenic [4, 5], the data of the present study emphasized the importance of investigating the joint effects of carcinogens and these drugs rather than studying the action of potent carcinogens alone.

REFERENCES

1. Conney AH, Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**: 317–366, 1967.
2. Conney AH and Burns JJ, Metabolic interactions among environmental chemicals and drugs. *Science* **178**: 576–586, 1972.

3. Kuntzman R, Jacobson M, Schneidman K and Conney AH, Similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in liver microsomes. *J Pharmacol Exp Ther* **146**: 280–285, 1964.
4. Haese WH and Bueding E, Long-term hepatocellular effects of hycanthone and of two other antischistosomal drugs in mice injected with *Schistosoma mansoni*. *J Pharmacol Exp Ther* **197**: 703–713, 1976.
5. Urman HK, Bulay O, Clayson DB and Shubik P, Carcinogenic effects of niridazole. *Cancer Lett* **1**: 69–74, 1975.
6. Omura T and Sato R, A new cytochrome in liver microsomes. *J Biol Chem* **237**: 1375–1376, 1962.
7. Williams CH and Kamin H, Microsomal triphosphopyridine nucleotide-cytochrome *c* reductase of liver. *J Biol Chem* **237**: 587–595, 1962.
8. Wiebel FJ and Gelboin HV, Aryl hydrocarbon (benzo(*a*)pyrene) hydroxylases in liver from rats of different age, sex and nutritional status. Distinction of two types by 7,8 benzoflavone. *Biochem Pharmacol* **20**: 1511–1515, 1975.
9. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin-phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
10. Kaul KL and Novak RF, Induction of rabbit hepatic microsomal cytochrome P-450 by imidazole: Enhanced metabolic activity and altered substrate specificity. *Arch Biochem Biophys* **235**: 470–481, 1984.
11. Koop DR, Crump BL, Nordblom GD and Coon MJ, Immunochemical evidence for induction of the alcohol-oxidizing cytochrome P-450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole and isoanizid. *Proc Natl Acad Sci USA* **82**: 4065–4071, 1985.
12. Ortiz de Montellano PR, Mico BA and Yost GS, Suicidal inactivation of cytochrome P-450. Formation of a heme–substrate covalent adduct. *Biochem Biophys Res Commun* **83**: 132–137, 1978.
13. Iyanagi T, Anan FK, Imai Y and Mason HS, Studies on the microsomal mixed-function oxidase system: redox properties of detergent-solubilized NADPH-cytochrome P-450 reductase. *Biochemistry* **17**: 2224–2230, 1978.
14. Long RK, Goldstein JA and Birnbaum LS. Effect of *o*-benzyl-*p*-chlorophenol on drug-metabolizing enzymes in rats. *Biochem Pharmacol* **35**: 613–620, 1986.
15. Mannering GJ, Sladek NE, Parli CJ and Shoeman DW, Formation of a new cytochrome P-450 hemoprotein after treatment of rats with polycyclic hydrocarbons. In: *Microsomes and Drug Oxidations* (Eds. Gillette JR, Conney AH, Cosmides GJ and Estabrook JR), pp. 303–330. Academic Press, New York, 1969.
16. Toftgard R, Nilson OG, Ingelman SM and Gustafsson JA, Correlation between changes in enzymatic activities and induction of different forms of rat liver microsomal cytochrome P-450 after phenobarbital, 3-methylcholanthrene and 16-cyanopregnenolone treatment. *Acta Pharmacol Toxicol* **46**: 353–361, 1980.
17. Evarts RP and Mostafa MH, Effects of indole and tryptophan on cytochrome P-450, dimethylnitrosamine demethylase and aryl hydrocarbon hydroxylase activities. *Biochem Pharmacol* **30**: 517–522, 1981.