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The effect of praziquantel on the hepatic activities of some drug-metabolizing enzymes in rabbits experimentally infected with *Schistosoma bovis*

H.A. ELSHEIKH*,‡
W.M. KHEIR*&
A.S. ABDULLAH†

*The Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Khartoum, PO Box 32 Khartoum North, Sudan, †The Department of Biomedical Science, Faculty of Biomedical and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia, ‡Correspondence: Department of Biomedical Sciences, Faculty of Biomedical and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia

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Schistosomiasis causes clinical and pathological manifestations comprising emaciation, diarrhoea, dehydration, anorexia, muscular weakness, signs of abdominal pain and anaemia (Saad *et al.*, 1980) and produces changes in the activity of enzymes indicating liver injury as previously reported in calves and mice (Rutkowski & Brucei, 1971; Mahmoud *et al.*, 1987). Moreover, experimental infection of mice with *S. mansoni* was shown to cause some effects on the hepatic activities of some enzymes responsible for drug biotransformation (Cha & Edwards, 1976; Cha *et al.*, 1980). This was highly suggestive of interference by the infection with the integrity of the endoplasmic reticulum, as it had a direct relationship with the activities of drug-metabolizing enzymes (Buymarzky *et al.*, 1978). Therefore, it was suggested that, as part of their mechanism of action, chemotherapeutic agents might possess the ability to repair the integrity of the endoplasmic reticulum. This repair can be well expressed by the restoration of the activities of the drug-metabolizing enzymes in treated animals. Accordingly, the effect of a therapeutic dose (40 mg/kg) of the schistosomicidal drug praziquantel, on the hepatic activities of some drug-metabolizing enzymes in rabbits experimentally infected with *S. bovis*, was investigated. The enzymes studied were aminopyrine N-demethylase and aniline 4-hydroxylase, which represent Phase I drug-metabolizing enzymes. Their activities were mediated by different cytochrome P-450 isoenzymes, and UDP-glucuronyltransferase which is regarded as an important Phase II enzyme. These enzymes were selected because their assay could be conducted with a high degree of precision using the available laboratory facilities.

The severity of the infection of rabbits with *S. bovis* was assessed by clinical signs, effect on the activities of the plasma enzymes sorbitol dehydrogenase (SD), glutamate dehydrogenase (GD) and aspartate aminotransferase (AST), faecal egg counts and by gross and histopathological changes in the liver and intestine.

This study was evaluated and approved by the Faculty Research Board of the Faculty of Veterinary Science at the University of Khartoum, which is responsible for the ethical use of experimental animals. Forty two young adult male rabbits (*Oryctolagus cuniculus*), of a local domestic breed, 6–7 months of age and weighing 1–1.2 kg were used. They were kept in clean disinfected pens at a temperature ranging between 25 and 30°C. They were fed on balanced feed and they had free access to drinking water. The animals were divided into seven groups (A, B, C_1, C_2, D_1, D_2 and E) of six animals per group. Rabbits in group A were kept as uninfected controls. Each animal in the remaining groups was infected percutaneously, in the flank region after shaving the fur over an area 6–8 cm in diameter, with 600 cercariae of *S. bovis*. The cercariae was produced under standard laboratory conditions from the snail *Bolinus africanus*. Rabbits in groups B, C_1, D_1 and E were slaughtered 6, 9, 13 and 16 weeks after infection, respectively. Animals in groups C_2 and D_2 were treated orally with praziquantel at a dose of 40 mg/kg 8 and 11 weeks after infection, and slaughtered 1 and 2 weeks following treatment, respectively. Control rabbits (group A) were killed 16 weeks after the start of the experiments together with the animals of group E.

Two to three millilitres of blood were collected from the ear vein in heparinized syringes 1 week before infection and at weekly intervals thereafter. Plasma was separated by centrifugation of blood at 900 ***g*** for 15 min. The collected plasma was stored at –20°C until analysed for protein concentration as described by Weichselbaum (1946), and the activities of the plasma enzymes SD, GD and AST according to the colorimetric methods of Ford (1967), Ford and Boyd (1962) and Reitman and Frankel (1957), respectively.

Faecal samples were taken before infection of rabbits for the detection of eggs of schistosoma and other internal parasites and continued weekly after infection for confirmation of *S. bovis* infection. Faecal examination was conducted by the modified Sheathers (1923) floatation technique and the modified sedimentation method described by Duwel and Reisenleiter (1984).

Rabbits were fasted for 18 h for the purpose of decreasing liver glycogen levels and to prevent loss of microsomes during the centrifugal isolation procedure (Dallner *et al.*, 1966). Soon after death, post-mortem examination was conducted in all rabbits. A small necropsy specimen was collected from the liver and the different parts of small intestine, placed in 10% formalin-saline, dehydrated, embedded in paraffin wax and cut at 5 μm thick sections and stained with haemotoxylin and eosin (H & E).

Immediately after slaughtering of the animals, pieces (5–7 g) of livers were obtained, quickly immersed in liquid nitrogen, wrapped in aluminium foil and stored in liquid nitrogen prior to analysis within 1 week. Weighed pieces of liver were homogenized in ice-cold isotonic KCl (0.15 M pH 7.4) by six to eight strokes of a motor-driven Teflon homogenizer to give 10–20% W/V homogenates. The crude homogenates were then centrifuged in a refrigerated centrifuge (UJIIKS cold centrifuge) at 4°C for 10 min at 10 000 ***g***. The microsomes were prepared for the estimation of protein concentration by the calcium aggregation method of Aitio and Vainio (1976). Protein was assayed in the crude homogenate, cytosolic and microsomal fractions of each liver sample by the method of Lowry *et al.* (1951) as modified by Miller (1959).

The activities of aminopyrine N-demethylase and aniline 4-hydroxylase were estimated spectrophotometrically by the determination of formaldehyde and p-aminophenol concentrations, respectively, as described by Mazel (1971). UDP-glucuronyltransferase activity was estimated spectrophotometrically by the method of Dutton and Storey (1962).

Before assay of these enzymes, preliminary experiments were carried out to determine the optimum conditions for estimations of enzymes activity, namely, substrate concentration, buffers pH, amount of homogenate and incubation temperature and time.

Student's *t*-test was used for statistical analysis and differences were considered significant when $P < 0.05$.

All the infected rabbits developed a classical picture of schistosomiasis (Rutkowski & Brucei, 1971; Saad *et al.*, 1980). Five to six weeks following infection, the rabbits started to show signs of dullness, weakness, loss of weight and had roughened fur. Schistosoma eggs were seen 4–6 weeks post-infection and thereafter until the end of the experiment. No schistosoma eggs were seen in the faeces of animals after treatment with praziquantel (groups C_2 and D_2).

The activities of plasma enzymes SD and GD were significantly increased, in the infected rabbits, by week 2 or 3 until week 13 ($P < 0.05$). These enzymes reached maximum levels 5–6 weeks post-infection, and then started to decline. The activity of AST was significantly increased ($P < 0.05$) 3–4 weeks post-infection until week 16, reaching a maximum level by week 6 or 7 post-infection. The plasma protein concentration was significantly decreased ($P < 0.05$) 5–6 weeks post-infection until the end of the experiment. In animals of groups C_2 and D_2, after treatment with praziquantel, the enzyme activities declined significantly until the time of slaughter.

The liver of rabbits in groups B, C_1, D_1 and E showed superficial necrotic foci. Moreover, rabbits in group E had livers with pale edges and large numbers of worms were seen in the mesenteric veins, while rabbits treated with praziquantel (groups C_2 and D_2) had small necrotic foci in the liver and only few stunted worms were reported in the mesenteric veins. No gross post-mortem changes were observed in other organs.

Histopathological sections prepared from the livers of rabbits in group B showed slight cirrhosis with characteristic newly formed bile ducts. Livers from rabbits in groups C_1 and D_1 showed schistosomal pigment engulfed in the Kupfer's cells and distinct granulomatous lesions around the portal area surrounded by inflammatory cells. Variable degrees of congestion and interlobular hyperplasia were seen in livers of groups C_2 and D_2. Liver sections from rabbits in group E showed marked centrilobular necrosis and the area around the portal vessels showed severe infiltration with leukocytes. Sections from the duodenum of rabbits representing groups B, C_1, C_2, D_1, D_2 and E showed variable degrees of submucosal infiltration with inflammatory cells.

The effect on rabbits of the experimental infection with *S. bovis* at various stages, and the effect of praziquantel treatment on protein concentration in liver homogenates and cytosolic and microsomal fractions are represented in Table 1. No significant changes were observed in protein concentrations other than a decrease in the concentration of microsomal and cytosolic proteins in groups B ($P < 0.01$) and C_1 ($P < 0.001$), respectively.

The activities of the drug-metabolizing enzymes aminopyrine N-demethylase and aniline 4-hydroxylase which represent Phase I drug-metabolizing enzymes were not similarly affected by *S. bovis* infection (Table 2). The activity of aminopyrine N-demethylase was significantly reduced 6–9 weeks post-infection, whereas the activity of aniline 4-hydroxylase was significantly increased from the ninth to the sixteenth weeks post-infection. These findings were not in agreement with the previous findings of Cha and Edwards (1976) and Cha *et al.* (1980) who reported a decrease in the activity of the two enzymes, in addition to differences in the time required for the change in the enzyme activities. The incompatibility in these findings might be attributed to differences in the Schistosoma species and the animal species used. The increase in the activity of aniline 4-hydroxylase might have been because of the enzyme activation rather than enzyme induction, since the amount of microsomal protein was not significantly increased. This activation might be an adaptive response to cope with the external insult, and/or the enzyme might have played a considerable role in the detoxification of the endogenous toxins produced by the parasite. In the present study, the activities of aminopyrine N-demethylase and UDP-glucuronyltransferase were significantly reduced (Table 2). Similar results were obtained by Cha and co-workers (1980). The decrease in enzyme activities may be because of the biochemical or morphological effects of the parasite on the liver cells. The appearance of cytochrome P-420 during some parasitic infections (Fascioliasis & hepatic amaebiasis) was indicative of the degradation of cytochrome P-450 to its catalytically inactive form (Tekwani *et al.*, 1988). Similarly phospholipases, proteases, and detergents such as deoxycholate, phenols and many heterocylic compounds were also reported to cause the degradation of

Table 1. The effect of experimental infection[1] of rabbits with *Schistosoma bovis* and praziquantel treatment[2] on the hepatic protein concentrations (mg/g) of the whole homogenate, cytosolic and microsomal fractions

	Control Group A (n = 6)	Infected[1] Group B (n = 6)	Group C_1 (n = 6)	Group C_2 (n = 6)	Group D_1 (n = 6)	Group D_2 (n = 6)	Group E (n = 6)
Whole homogenate	168.069 ± 13.256	184.009 ± 9.020	186.328 ± 3.998	168.330 ± 11.062	171.201 ± 10.181	186.672 ± 3.656	168.069 ± 13.256
Cytosolic	94.688 ± 4.663	87.404 ± 3.745	72.845 ± 1.391**	92.341 ± 2.371	87.022 ± 1.469	83.620 ± 5.339	94.688 ± 4.663
Microsomal	20.906 ± 2.090	13.845 ± 1.923*	18.877 ± 0.299	18.211 ± 1.113	18.412 ± 0.188	18.640 ± 0.035	20.906 ± 2.090

Values in the table are means ± SEM.
[1]Each animal was infected percutaneously in the flank region with 600 cercariae of *S. bovis*.
[2]Praziquantel (40 mg/kg) was given orally to each of the rabbits of groups C_2 and D_2 8 and 11 weeks after infection, respectively, and animals were then killed 1 and 2 weeks later, respectively.
*$P < 0.01$ compared to control values.
**$P < 0.002$ compared to control and group C_2 values.

Table 2. The effect of experimental infection[1] of rabbits with *Schistosoma bovis* and praziquantel treatment[2] on the hepatic activities of aminopyrine N-demethylase, aniline 4-hydroxylase and UDP-glucuronyltransferase

	Control Group A (n = 6)	Infected[1] Group B (n = 6)	Group C_1 (n = 6)	Group C_2 (n = 6)	Group D_1 (n = 6)	Group D_2 (n = 6)	Group E (n = 6)
Aminopyrine N-demethylase[3]	9.423 ± 0.856	4.389 ± 0.605**	6.631 ± 0.043*	11.533 ± 0.052*[a]	4.668 ± 0.254**	9.027 ± 0.213[c]	9.510 ± 1.080
Aniline 4-hydroxylase[3]	0.446 ± 0.041	0.538 ± 0.026	0.491 ± 0.067	0.791 ± 0.011***[b]	0.560 ± 0.017*	0.673 ± 0.017**[c]	0.877 ± 0.011***
UDP-glucuronyl-transferase[3]	3.816 + 0.183	1.249 ± 0.187**	3.119 ± 0.088	3.451 ± 0.039[b]	2.036 ± 0.013**	2.840 ± 0.009**[c]	4.329 ± 0.375

Values in the table are means ± SEM.
[1]Each animal was infected percutaneously in the flank region with 600 cercariae of *S. bovis*.
[2]Praziquantel (40 mg/kg) was given orally to each of the rabbits of groups C_2 and D_2 8 and 11 weeks after infection, respectively, and animals were then killed 1 and 2 weeks later, respectively.
[3]Enzyme activities are expressed as n mole/mg of microsomal protein/minute.
*$P < 0.05$ compared to control values.
**$P < 0.01$ compared to control values.
***$P < 0.001$ compared to control values.
[a]$P < 0.001$ compared to group C_1 value.
[b]$P < 0.001$ compared to group D_1 values.
[c]$P < 0.01$ compared to group C_1 values.

cytochrome P-450 to P-420 (Omura & Sato, 1964). The deposition of schistosomes pigment in the liver of the infected host could have conceivably been responsible for the marked reduction of cytochrome P-450 levels as reported by Cha *et al.* (1980), that similar factors might have been generated during these parasitic infections.

In the present study, rabbits experimentally infected with *S. bovis* and treated with praziquantel (groups C_2, and D_2) had a significant increase in the activities of the three drug-metabolizing enzymes compared to those killed 9 and 13 weeks post-infection which did not receive praziquantel (Table 2). As the drug has no inductive effect on these enzymes in non-infected treated rabbits (Kheir *et al.*, 1995), the increase produced by praziquantel treatment might be because of the destruction of the schistosomes by the drug as suggested by previous workers (Shaheen *et al.*, 1989). A repairing process in the liver tissue might have contributed to the increase in the activities of the drug-metabolizing enzymes. A further support to this explanation is the decrease in drug-metabolizing enzymes activity which might have been because of the deposition of schistosomes pigment in the liver of infected animals as suggested by Cha *et al.* (1980). The removal of the parasite by praziquantel chemotherapy would prevent the production of pigment that might have adversely affected the microsomal enzymes activity.

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