J. Pharm. Pharmacol. 1983, 35: 729–733
Received February 7, 1983

Impairment of drug metabolism by the liver in experimental fascioliasis in the rat

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Fascioliasis has been produced in the rat by an oral administration of 20 metacercariae of *Fasciola hepatica*. Hepatic microsomal cytochrome P450 and b5 contents and both aminopyrine demethylase and aniline hydroxylase activities have been measured during the course of the experimental distomiasis. The cytochrome P450 concentration and microsomal drug metabolizing enzymes generally fell by weeks 3 to 8 post-infestation and recovered to normal values thereafter. For the same period, both the histoenzymatically assayed liver cytochrome oxidase and arylsulphatase activities were reduced whereas there were correlated increases in glutamic pyruvic and glutamic oxaloacetic plasma transaminases. Tissue inflammation and destruction provoked by young histophagous migrating flukes could be responsible for these changes that have already been observed in several hepatic diseases. The possible influence of naturally-induced fasciolasis on liver drug metabolism is discussed.

Liver diseases such as acute and chronic hepatitis, cirrhosis, cholestasis and obstructive jaundice generally lead to an impairment of drug metabolizing activities (Jenner & Testa 1981). In spite of the frequent occurrence of liver parasitism by *Fasciola hepatica* in both man and breeding animals, there has been a lack of information about the effect of this infection on hepatic drug biotransformation systems.

Therefore we developed a rat model for fascioliasis so that we could investigate the effect of this condition on the level of cytochrome P450 and some enzymatic activities, i.e. aniline hydroxylase, aminopyrine demethylase, cytochrome oxidase and arylsulphatase, as determined by means of either biochemical or histochemical methods. This study was designed to discriminate the various stages of fluke infestation and to attempt correlations between alterations in liver enzyme activities and changes in plasma components that are recognized as possible indicators of liver injury in animals with experimental fascioliasis.

METHODS

Treatment of animals

Sprague Dawley male rats, about 200 g, were randomly distributed into control or infested groups of 6 rats and housed in cages of 3 animals. Food (UAR alimentation, Villemoisson, France) and drinking water were freely available. Each infected rat received by gastric tube 20 metacercariae of *Fasciola hepatica* suspended in a 1% Tween aqueous solution. The development of the condition was observed clinically and by means of haematological and biochemical studies of blood samples. Parallel studies were carried out on uninfested control animals. 12 animals (6 infested and 6 uninfested rats) were killed at the end of each week for 8 weeks and also by weeks 10, 12 and 14 after the infestation. The abdomen was opened and the blood drawn by puncture through the abdominal aorta. The liver was removed immediately, freed of extrahepatic tissue, blotted free of excess moisture and weighed (0–4 °C).

As described by De Duve et al (1955), a randomized 5 g sample of liver was homogenized with 20 ml of ice-cold 0·1 M Na/K phosphate buffer, pH 7·4, in a glass Potter homogenizer with a Teflon pestle. The homogenate was centrifuged at 10 000*g* for 20 min in a Jouan K 101 refrigerated centrifuge. The supernatant fraction was then centrifuged for 1 h at 105 000*g* in the Ti50 rotor of Beckman L550 ultracentrifuge. The microsomal pellet was suspended in an ice-cold solution consisting of potassium chloride (1·15%) and Na/K phosphate buffer (0·1 M, pH 7·4).

Assays

The number of *Fasciola* eggs in the rat faeces was counted as described by Campbell et al (1978). Adult flukes within the common bile duct of infested rats

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killed in weeks 6 to 14 were counted. Total leucocyte count, erythrocyte count and packed cell volume were obtained with the use of an electronic Coulter-counter device. The activities of plasma glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were estimated at 37 °C by the method of Reitman & Frankel (1957) by means of the Biochemica Test Combination (Boerhinger Mannheim, ref. 125903 and 125881). The microsomal protein content was determined with bovine serum albumin as the reference protein (Lowry et al 1951). The content of cytochrome P450 was measured from the difference spectra between a microsomal sample reduced with sodium dithionite and one gassed with CO (Omura & Sato 1964). The content of cytochrome b5 was calculated from the sodium dithionite-reduced sample spectra. Aniline hydroxylase and aminopyrine demethylase hepatic microsomal activities were determined according to Mazel (1971). The formation of formaldehyde from demethylated aminopyrine was estimated by the method of Nash modified by Cochin & Axelrod (1959).

Histology

To search for representative lesions caused by young flukes on liver parenchyma and mature flukes in bile ducts, liver tissue samples were fixed in 10% buffered formalin and further dehydrated with ethanol, embedded in paraffin, sectioned at a thickness of 5 μm and stained with haematein and eosin. Enzyme activities were localized on unfixed liver tissue. Samples were immediately frozen in dry ice and cut at 15 μm in a cryostat (−15 °C). The cytochrome oxidases activity was determined by the naphthol-amine technique of Burstone (1959) using diphenylamine and 1-hydroxy-2-naphthoic acid as coupler. The incubation time was 2·5 h. Sodium azide was used as inhibitor at a concentration of 5 mM. The arylsulphatase activity was determined by the method of Rutenburg et al (1952) using 6-benzoyl-2-naphthyl sulphate (potassium salt) and fast blue B salt as the post coupling azo dye with an incubation time of 4 h.

Analysis of data

Differences between the values for each time group of infested and control animals were analysed by using an analysis of variance with interaction. They were further assayed for individual differences by Student's *t*-test and for correlation between the biochemical parameters.

RESULTS

4 to 5 weeks after the beginning of the experiments, fluke eggs were recovered in faeces of infested rats. In those animals killed in weeks 6 to 14, 4·6 ± 0·7 flukes were located within the common bile duct, corresponding to the primary infection with 20 metacercariae. At autopsy, lesions characteristic of long-standing fascioliasis were present in the livers of all infected rats. When the infestation started, the liver appeared to be permeated by dark haemorrhagic streaks and foci, lesions were more marked from weeks 4 to 8. Fibrosis extended into several areas of the liver lobes. The bile duct was enlarged and fibrosed particularly from 6 weeks after infestation.

The haematological investigation demonstrated no significant differences between control and infested rats for both leucocyte and erythrocyte counts and packed cell volumes. There were marked

Table 1. Changes in plasma and liver parameters during the course of the experimental fascioliasis in rats (mean ± s.e.m. of six rats).

	Plasma		Liver	
Weeks post infestation	GOT (units ml^{-1})	GPT (units ml^{-1})	liver/body wt ratio (%)	microsomal proteins ($mg\,g^{-1}$)
controls	57·8 ± 6·4	66·3 ± 7·7	3·86 ± 0·21	34·3 ± 4·7
1	48·3 ± 6·0	51·6 ± 4·2	4·35 ± 0·37	30·7 ± 9·0
2	56·3 ± 2·4	76·9 ± 9·6	4·72 ± 0·51	36·8 ± 2·4
3	103·0 ± 10·5*	102·3 ± 12·8*	3·85 ± 0·24	37·1 ± 0·9
4	147·0 ± 11·8*	171·7 ± 18·0*	3·98 ± 0·30	31·5 ± 2·4
5	124·2 ± 14·8*	128·5 ± 16·6*	4·07 ± 0·37	38·7 ± 2·0
6	99·8 ± 23·9	104·0 ± 15·4*	4·32 ± 0·17	30·3 ± 3·1
7	66·2 ± 7·7	81·2 ± 16·6	4·00 ± 0·27	29·5 ± 5·2
8	68·2 ± 13·7	67·2 ± 20·4	3·97 ± 0·40	31·5 ± 6·2
10	60·2 ± 11·7	65·1 ± 12·2	3·41 ± 0·20	28·4 ± 5·8
12	70·5 ± 11·9	78·0 ± 13·7	3·54 ± 0·10	31·6 ± 3·5
14	55·2 ± 12·2	56·2 ± 6·3	3·53 ± 0·37	30·8 ± 3·8

The results have been analysed by Student's *t*-test and * indicates a statistical difference ($P < 0·05$) between the control and pathological data.

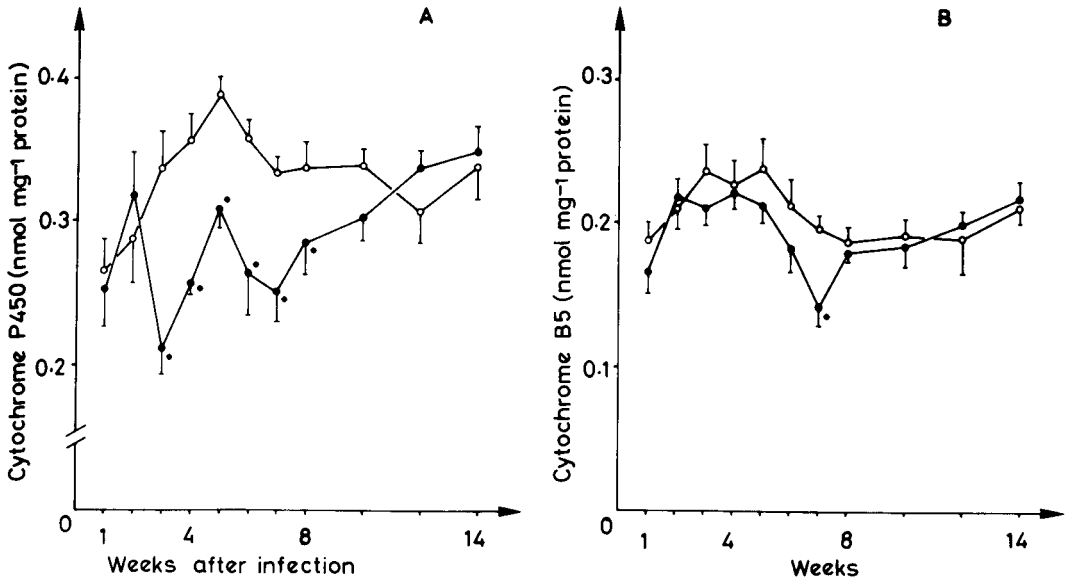

FIG. 1. Cytochrome P450 (A) and b5 (B) content in liver microsomes of rats during the course of the disease. Each value represents the mean ± s.e.m. obtained from 6 rats. Statistical differences ($P < 0{\cdot}05$) between control (○) and infested (●) rats compared by Student's *t*-test are indicated by asterisks.

increases in GOT and GPT plasma activities by weeks 3 to 5 and 6 (Table 1). However, throughout the experimental period, there was no change in liver to body weight ratio or liver microsomal protein concentration. Analysis of variance clearly demonstrated decreases in cytochrome levels and microsomal enzyme activities. All these decreases were correlated with an increase in plasma transaminase concentrations. The cytochrome P450 concentration appeared significantly lower in infested rats com-

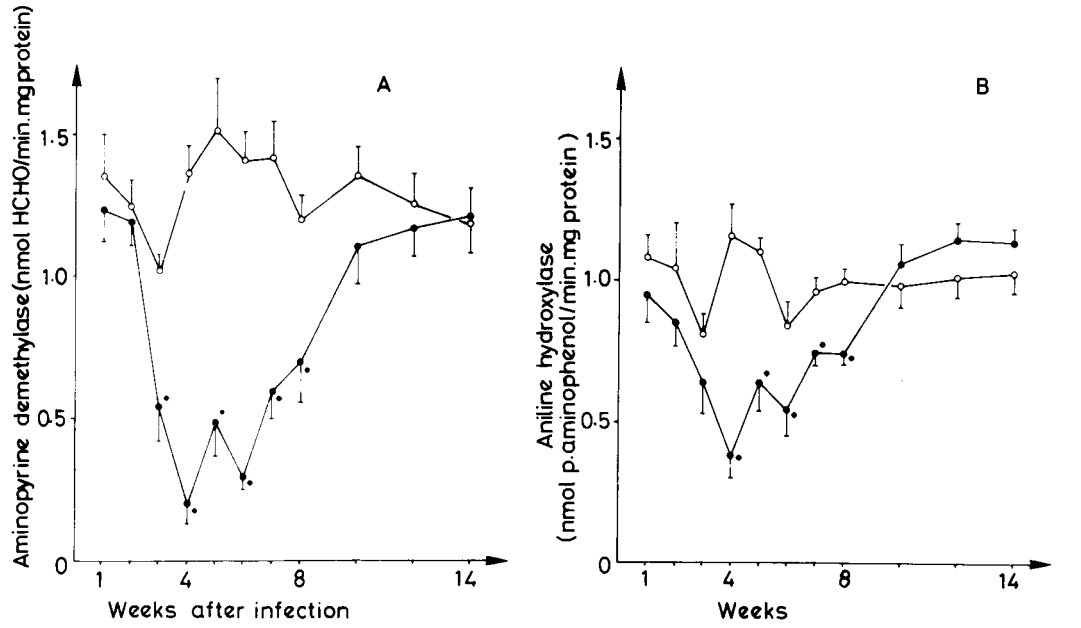

FIG. 2. Changes in the activity of aminopyrine demethylase (A) and aniline hydroxylase (B) in liver microsomes of rats. Each value represents the mean ± s.e.m. obtained from 6 animals. Statistical differences ($P < 0{\cdot}05$) between control (○) and infested (●) rats compared by Student's *t*-test are indicated by dots.

pared with controls between 3 to 8 weeks post infection (Fig. 1) whereas cytochrome b5 was only decreased in the livers of rats at 5 weeks post infection. Percent changes in the concentration of the drug metabolizing microsomal enzymes were similar to those in cytochrome P450 values (Fig. 2). For instance, by weeks 4 and 8, the activity of aminopyrine *N*-demethylase decreased 85 and 42%; in the same time, aniline hydroxylase decreased 67 and 27% respectively. In all cases, microsomal enzyme activities and concentrations of cytochromes recovered to normal values 10 weeks after infection and thereafter.

As observed by histological methods, the early infestation began by venous and sinusoid blood stasis, followed by an eosinophilic infiltration of portal spaces, numerous young flukes could be observed microscopically within tortuous migration tunnels filled with blood, debris and young parasites. In older stages, eosinophilic infiltration, phagocytes and giant cells were present in the tunnels. Two weeks later, no parasite was seen in the liver parenchyma which appeared divided into regeneration nodules lined by connective tissue septa as seen in portal cirrhosis. In some cases, the liver parenchyma was restored *ad integrum* and numerous mitotic cells were observed. In control rats, cytochrome oxidases and arylsulphatases showed normal activities as illustrated in Fig. 3A (dark ring-shaped areas in the parenchyma surrounding unstained portal veins). In infested rats, the enzymatic activities were markedly reduced (Fig. 3B) and could even be negative.

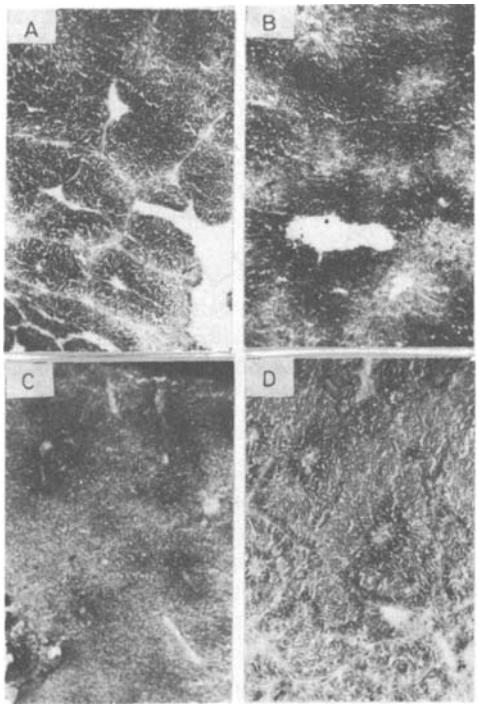

FIG. 3. Cytochrome oxidase activity in rat liver (×50). A. Control liver: the strong enzyme activity is marked by dark ring-shaped areas around the unstained portal veins. B. Infested liver: weak and diffuse activity enzyme activity is shown by limited dark areas in 5 weeks-infected rats. Arylsulphatases activity (×50). C. Control liver: as for cytochrome oxidase activity, large dark areas of activity are localized around the light portal spaces. D. Infested liver (5 weeks): the activity is limited to small rings in the nodule centre and to narrow bands lining the fibrous nodular septa.

DISCUSSION

The pathological signs seen in experimentally infested rats correspond to data reported in previous studied. Thus, fluke counts measured within the bile duct appear in agreement with 4·3 to 4·7 flukes recovered in rats infested with 25 metacercariae (Kelly & Campbell 1979). Post mortem and histological findings such as blood stasis, portal eosinophilic infiltration and tortuous migration tunnels are representative of distomiasis (Jubb & Kennedy 1970). Increases in GOT and GPT activities have already been described in experimentally infected rabbits with *Fasciola hepatica* (Le Bars & Banting 1979) or in the case of massive infestation in lambs (Pullan et al 1970). On the other hand, as previously demonstrated in rabbits (Le Bars & Banting 1979), the levels of transaminases returned to within normal limits 10 weeks after the single infestation. On this basis, the present experimental model is a suitable one and may be used to study impaired drug metabolism.

There is no significant change in either liver to body weight ratio or microsomal protein levels in infested rats; such results are generally obtained in most hepatic diseases.

The major finding is the significant and transient decrease in both microsomal cytochrome concentrations and drug metabolizing activities in experimental fascioliasis. The reduction in cytochrome P450 has been reported for many acute and chronic liver diseases including hepatitis or hepatic tumours in man (Jenner & Testa 1981). The decreases appear generally significant within the period 3 to 8 weeks after infestation. This delay corresponds to the migration of young histophagous flukes through the liver parenchyma and their development within bile ducts. It is well known that the acute hepatic lesions

provoked by the wandering flukes are basically traumatic but there is an element of coagulation necrosis which is possibly related to toxic excretions of the flukes (Jubb & Kennedy 1970). Thus, the necrosis may lead to localized ischaemic areas in which activities of enzymes and coenzymes associated with membranes of the endoplasmic reticulum have been shown to be affected (Ferrero et al 1978). It is relevant to parallel the lack of incidence of experimental ischaemia in rat on microsomal cytochrome b5 and the slight change (only by week 7) of this coenzyme in the course of this experimental fascioliasis.

Although the activities of the cytochrome oxidases and arylsulphatases assayed by means of histoenzymology did not correspond exclusively to specific drug metabolizing activities, they were generally lowered during the period when decreases in biochemically determined microsomal enzymes and coenzymes occurred (by weeks 3 to 8 post-infestation). This demonstrates the extent of the liver cellular damage induced by the fluke infection. On the other hand, the negative correlation obtained for weeks 3 to 6 between microsomal enzyme activities and plasma transaminases may be related to the inflammatory state of liver and to the tissue destruction provoked by migrating young flukes. By week 10 and thereafter, both the change in parasite physiology from histophagous to haematophagous states and the liver regeneration observed histologically should compensate for the previous traumatic damage and lead to recovery of normal liver and blood biochemical values.

With natural fascioliasis however, the repetitive infection may lead to more lasting changes in the hepatic drug metabolizing activities. Finally, as infested liver cells lose much of their capacity for drug metabolism, it is reasonable to suppose that, in analogous situations, e.g. human and animal distomiasis, the capacity of the liver for handling drugs and xenobiotics generally might also be reduced and consequently there would be a need for the adjustment of drug dosages.

Acknowledgements

The authors appreciate the skilful assistance of Dr Charpenteau (Laboratoire de Biométrie INRA, Toulouse) and Mrs Perdu (Laboratoire des Additifs Alimentaires INRA, Toulouse) in statistical and biochemical analysis and that of Mrs Eeckhoutte and Mrs Bories in technical assistance.

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