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# Characterization of the microsomal cytochrome P-450 species inhibited in rat liver in the course of fascioliasis

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Experimentally induced fascioliasis has been described to decrease significantly hepatic mixed function oxidase in rats 3–8 weeks after infestation [1, 2]. More recently, the incidence of this parasitism was investigated in rats infected for 5 weeks and untreated or pretreated with either phenobarbital, 3-methylcholanthrene, β-naphthoflavone or arochlor 1254 [3]. This consisted in decreasing liver microsomal P-450,* aminopyrine *N*-demethylase, aniline hydroxylase and mutagenic activation of cyclophosphamide which were specifically induced by both PB and arochlor [4].

The existence of several different isozymes of cytochrome P-450 differing in substrate specificity, aminoacid sequence, electrophoretic mobility and immunological properties is now well-established [5]. In the present investigation, we have compared the isozymes of P-450 more probably destroyed or repressed in the course of experimental fascioliasis in untreated and PB or MC induced rats, on the basis of substrate specificity, sensitivity to different inhibitors and immunological properties. In order to recognize a possible dual effect of *Fasciola hepatica* infection on microsomal cytochrome P-450 species in the course of parasitic disease, all studies were carried out in both 3 and 6 week-infected rats, since at these stages, parasitism was characterized by respectively the beginning and the end of the histophagous migration of juvenile larvae through the liver parenchyma [6].

## *Materials and methods*

Phenobarbital sodium, 3-methylcholanthrene, metyrapone, α-naphthoflavone and electrophoresis reagents were bought from Serva (Heidelberg, F.R.G.). Peroxidase conjugated immunoglobulins were purchased by Dako (Copenhagen, Denmark). All other chemicals were the highest quality available. Distilled deionized water was used in all studies.

In the study of induction in *Fasciola hepatica* infected animals, male Sprague–Dawley rats (140–160 g) were randomly distributed into control or infested groups of 9 rats and housed in cages of 3 or 4 animals. Each infected rat received by gastric tubing 20 metacercariae of *Fasciola hepatica* suspended in a 1% Tween aqueous solution. Parallel studies were carried out on uninfected control animals receiving the same solution without metacercariae. By weeks 3 and 6 after the infection, PB (80 mg/kg) dissolved in saline was administered (i.p.) daily for 3 days; MC dissolved in corn oil was injected by the same route at a single dose of 80 mg/kg. PB- and MC-treated rats were killed 24 and 48 hr respectively after the last injection.

After killing, hepatic microsomes were prepared by differential ultracentrifugation and stored as previously described [2]. The microsomal protein concentration was determined according to Lowry *et al.* [7] with bovine albumin as the standard. Cytochrome P-450 was measured in microsomes as described by Omura and Sato [8]. Aminopyrine *N*-demethylase and benzphetamine *N*-demethylase, activities were measured by the Nash reaction and ethoxycoumarin *O*-deethylase was determined by direct fluorimetry [9]. This reaction was also measured in presence of α-naphthoflavone [0.01 mM].

UT-A, the main constitutive cytochrome P-450 form, PB-B the main phenobarbital inducible form and BNF-B the main β-naphthoflavone inducible form were purified as previously described [10, 11]; in those papers, $A_2NI$ corresponded to UT-A, B2PB to PB-B and B2MC to BNF-B. They were shown to be identical to those initially described [5] by western blotting [12]. Antibodies were raised in female New Zealand rabbits. Anti-UT-A was purified by immunoaffinity chromatography, it recognized in "western blots" male rat liver microsomes as a single band. Monoclonal anti-PB-B [13] recognized microsomes from PB-treated rats as a single band in "western blots". Anti-BNF-B were those described [11] and recognized liver microsomes as two bands, one corresponding to BNF-B and another one corresponding to ISF-G, the major form induced by isosafrole [5]. It was not possible to measure this form because of the lack of pure antigen.

The immunoquantification was performed as previously described [12] and the statistical analysis was carried out using analysis of variance followed by the Dunnett test [14].

## *Results and discussion*

At autopsy, lesions characteristic of fascioliasis [2] were present in livers of all infected rats. As reported in Table 1, the sum of immunochemically determined specific contents of the three P-450s was greater than the conventional spectral measurement, which is in agreement with Dannan *et al.* [15].

Total cytochrome P-450 was decreased in untreated infected animals because of the decrease in UT-A form which is predominant in such untreated rats [5]. The slight decrease in PB-treated infected animals in comparison with corresponding controls would correspond to the decrease in $P\text{-}450_{UT\text{-}A}$ which was lowered by treatment with PB. Since this form is much more decreased, whereas $P\text{-}450_{BNF\text{-}B}$ becomes the essential form in MC-treated rats [15], fascioliasis provoked no change in these animals. The invariability of both $P\text{-}450_{PB\text{-}B}$ and $P\text{-}450_{BNF\text{-}B}$ inducibility in the course of fascioliasis leads us to reject the hypothesis [3] that incidence of fascioliasis could be due to a particular localization of tissue damages in relation to the specific localization of cytochrome P-450 isozymes within the liver lobule. The high sensitivity of aminopyrine *N*-demethylase

* Abbreviation used: P-450, cytochrome P-450; MC, 3-methylcholanthrene; PB, phenobarbital; BNF, β-naphthoflavone; ISF, isosafrole; UT-A: P-450 isozyme from untreated rats (form A).

Table 1. Effects of inducers and fascioliasis on hepatic cytochrome P-450 concentrations in rats

| Inducer | Weeks postinfection | nmoles Cytochrome P-450/mg microsomal proteins | | | |
|---|---|---|---|---|---|
| | | Total | UT-A | Isozyme PB-B | BNF-B |
| Untreated | Controls | 0.58 (8) | 0.92 (2) | 0.14 (4) | 0.26 (4) |
| | 3 | 0.33 (9)* | 0.03 (2)* | 0.00 (4)* | 0.23 (3) |
| | 6 | 0.35 (9)* | 0.24 (2)* | 0.00 (4)* | 0.19 (3) |
| PB | Controls | 1.06 (9)† | 0.95 (2) | 0.73 (6) | 0.10 (3) |
| | 3 | 0.85 (9)* | 0.13 (2)* | 0.74 (6) | 0.08 (2) |
| | 6 | 0.76 (9)* | 0.25 (2)* | 0.66 (6) | 0.11 (2) |
| MC | Controls | 0.80 (9)† | 0.87 (2) | 0.15 (3) | 0.64 (2) |
| | 3 | 0.69 (9) | 0.11 (2)* | 0.06 (3)* | 0.83 (2) |
| | 6 | 0.72 (9) | 0.00 (2)* | 0.00 (3)* | 0.87 (3) |

The number of animals used is given in parentheses.
* Significantly different from corresponding uninfected controls, $P < 0.05$.
† Significantly different from corresponding untreated controls, $P < 0.05$.

Table 2. Effects of inducers and fascioliasis on hepatic drug metabolizing activities in rats (mean ± S.E.M.)

| Inducer | Weeks postinfection | Aminopyrine *N*-demethylase (nmol/mg × min) | Benzphetamine *N*-demethylase (nmol/mg × min) | Ethoxycoumarin *O*-deethylase Activity (nmol/mg × min) | Ethoxycoumarin *O*-deethylase Inhibition by α-naphthoflavone (%) |
|---|---|---|---|---|---|
| Untreated | Controls | 0.44 ± 0.05 | 1.50 ± 0.18 | 0.18 ± 0.02 | 38.8 ± 4.3 |
| | 3 | 0.06 ± 0.04* | 0.60 ± 0.15* | 0.12 ± 0.02 | 44.4 ± 7.6 |
| | 6 | 0.06 ± 0.03* | 0.70 ± 0.16* | 0.15 ± 0.03 | 54.0 ± 8.4 |
| PB | Controls | 0.91 ± 0.09† | 2.16 ± 0.18† | 0.41 ± 0.05† | 21.2 ± 2.5† |
| | 3 | 0.34 ± 0.04* | 2.32 ± 0.24 | 0.45 ± 0.05 | 29.0 ± 5.8 |
| | 6 | 0.27 ± 0.06* | 2.41 ± 0.30 | 0.43 ± 0.05 | 31.2 ± 4.1 |
| MC | Controls | 0.29 ± 0.04† | 0.96 ± 0.11† | 0.95 ± 0.14† | 67.3 ± 3.9† |
| | 3 | 0.02 ± 0.01* | 0.63 ± 0.11 | 1.25 ± 0.10* | 68.2 ± 4.6 |
| | 6 | 0.08 ± 0.03* | 0.47 ± 0.12* | 1.13 ± 0.15 | 71.1 ± 5.2 |

* Significantly different from corresponding uninfected controls, $P < 0.05$.
† Significantly different from corresponding untreated controls, $P < 0.05$.

(Table 2) to fascioliasis [1–3] could be related to the strong participation of the P-450$_{UT-A}$ form to this activity which was only induced by phenobarbital in our experiment. Since benzphetamine *N*-demethylase is more largely dependent on the P-450$_{PB-B}$ form than P-450$_{UT-A}$ or P-450$_{BNF-B}$ [5], this activity was not affected in PB-treated rats in which the selectivity-induced P-450$_{PB-B}$ form was not sensitive to fascioliasis. In contrast, in uninduced or MC-treated animals, the fluke infection destroyed isozyme P-450$_{UT-A}$ and consequently suppressed this monooxygenase activity. P-450$_{BNF-B}$ has been described as the main form active in catalyzing ethoxycoumarin *O*-deethylation [11]; for that reason fascioliasis provoked no repressive incidence on this activity whatever the pretreatment given because of its failure to inhibit P-450$_{BNF-B}$. This conclusion may also be applied to the inhibition of ethoxycoumarin *O*-deethylase by α-naphthoflavone which has been recognized as a P-450$_{BNF-B}$-dependent process [16]. In the case of MC-treated controls, aminopyrine *N*-demethylase and benzphetamine *N*-demethylase were lowered in comparison with untreated controls. This could be due to a reduced catalytic activity P-450$_{UT-A}$ related to competition with the other induced forms whereas the MC-induced form, P-450$_{BNF-B}$, did not participate substantially in these activities.

In conclusion, the correlations obtained in this study between enzyme activities and immunological estimation of three main forms of cytochrome P-450 demonstrate the specific incidence of fascioliasis on the P-450$_{UT-A}$ form and on biotransformation pathways in which this isozyme would be involved. The reasons for such a specificity could be related to the specification of toxic excretions of the flukes in the course of their histophagous migration through the liver parenchyma [4], these toxic-excretory products could be present in the microsomal fractions likely to contain microsomes from the larvae. In other respects, such a mixed infective plus inductive process could be used in studying the influence of one isozyme in microsomes after destruction of P-450$_{UT-A}$. This system could also bring new information in comparison with reconstituted systems in which reductases are very high, membrane are not really physiological and in which there is generally not competition between isozymes.

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