Biochemical Pharmacology, Vol. 41, No. 10, pp. 1513–1519, 1991
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

DIFFERENTIAL EFFECT OF BILIARY AND MICRONODULAR CIRRHOSIS ON OXIDATIVE DRUG METABOLISM

IN VIVO–IN VITRO CORRELATIONS OF DEXTROMETHORPHAN METABOLISM IN RAT MODELS

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(*Received* 8 *August* 1990; *accepted* 15 *December* 1990)

Abstract—Oxidative drug metabolism is impaired in liver cirrhosis; it is unclear, however, whether this depends on the etiology of cirrhosis. Therefore, we studied the metabolism of dextromethorphan in two rat models: biliary cirrhosis induced by bile duct ligation and micronodular cirrhosis induced by chronic exposure to CCl_4/phenobarbital. Results were compared with aminopyrine N-demethylation assessed by a breath test *in vivo*; the latter was reduced to a similar extent in biliary (−41%) and micronodular (−37%) cirrhosis compared to controls. In contrast, clearance of dextromethorphan was significantly ($P < 0.001$) reduced in biliary (25.4 ± 5.3 mL/min/kg) but not in micronodular cirrhosis (48.6 ± 15.6) as compared to controls (62.2 ± 16.2). Intrinsic clearance of dextromethorphan *in vitro* was reduced by 95% and 63% in biliary and micronodular cirrhosis, respectively ($P < 0.001$ vs controls). It correlated with dextromethorphan clearance *in vivo* ($r = 0.68$, $P < 0.001$) whereas correlation with aminopyrine N-demethylation was weak ($r = 0.42$, $P < 0.05$). Our results demonstrate a differential effect of biliary and micronodular cirrhosis on isoenzymes responsible for aminopyrine and dextromethorphan demethylation.

Liver cirrhosis impairs drug metabolism in animal models [1–4] and in man [5–8]. It is not clear, however, whether biliary cirrhosis has the same effect on microsomal functions as micronodular cirrhosis. Recent studies in our laboratory using bile duct ligated rats as a model for biliary cirrhosis and rats rendered cirrhotic by chronic exposure to CCl_4 and phenobarbital as a model for micronodular cirrhosis showed a differential effect on hepatic microsomes. Although the aminopyrine breath test was similarly impaired in both models, hepatic cytochrome P450 content was significantly more reduced in biliary than in micronodular cirrhosis [2, 9].

In order to further elucidate the differential effects of biliary and micronodular cirrhosis on microsomal functions, drug metabolism was probed *in vivo* and *in vitro* in rat models of biliary and micronodular cirrhosis using dextromethorphan as test compound. Dextromethorphan is a widely used antitussive agent with low toxicity [10–12]; it has been shown to be a useful probe to evaluate the debrisoquine-type hydroxylator phenotype in man [13] and in an animal model [14]. We chose it as a test compound to assess hepatic microsomal function for the following reasons: dextromethorphan and its major two metabolites undergo four different demethylation reactions, which can be measured simultaneously in microsomal incubations by a recently developed simple HPLC assay [14]. In the rat, the major metabolic pathways involve O-demethylation of dextromethorphan to dextrorphan. This compound is further N-demethylated to hydroxymorphinan. A second important pathway involves N-demethylation of dextromethorphan to methoxymorphinan, which is further O-demethylated to hydroxymorphinan (Fig. 1; [14]).

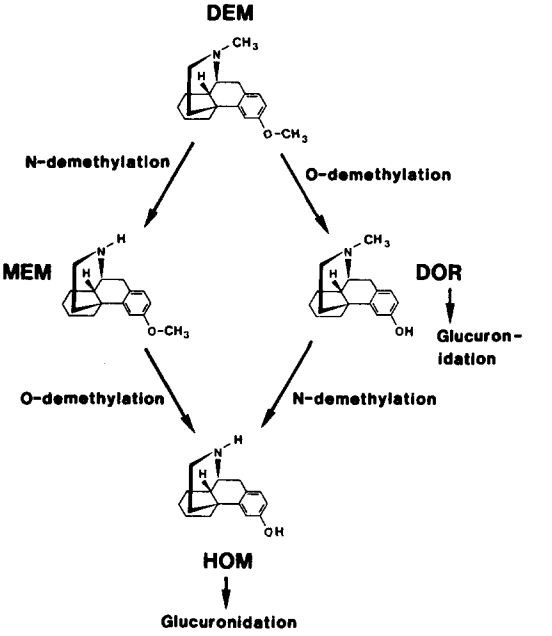

Fig. 1. Metabolic pathways of dextromethorphan (DEM) to dextrorphan (DOR); methoxymorphinan (MEM) and hydroxymorphinan (HOM).

The aim of this study was, therefore, to assess the

Table 1. Characteristics of biliary (BDL), micronodular (CCL) cirrhotic and control (CTR) rats; $\bar{x} \pm SD$ are shown

	BDL (N = 5)	CCL (N = 7)	CTR (N = 11)
Body weight (g)	538 ± 99	601 ± 45	645 ± 76*
Liver weight (g)	31.9 ± 13.7	21.3 ± 4.7	21.3 ± 3.7*
Spleen weight (g)	2.6 ± 1.2	2.6 ± 0.5	1.3 ± 0.3†‡
Serum bile acids (mol/L)	58.4 ± 43.3	5.9 ± 4.2	0.6 ± 0.4‡‖
Serum ALAT (I.U./L)	265 ± 122	152 ± 105	62.1 ± 23.1†§
Ascites (mL)	12 ± 9	10 ± 17	0
Portosystemic shunting (%)	9.3 ± 7.2	3.9 ± 4.2	0.01 ± 0.03†‡
ABT_{AUC} (% dose)	17.0 ± 6.4	19.0 ± 4.8	28.9 ± 5.2†‡

* $P < 0.05$ BDL vs CTR; † $P < 0.001$ BDL vs CTR; ‡ $P < 0.001$ CCL vs CTR; § $P < 0.01$ CCL vs CTR; ‖ $P < 0.01$ BDL vs CCL.

influence of two models of liver cirrhosis on demethylation of dextromethorphan *in vivo* and *in vitro*. The results were compared with the aminopyrine breath test, a standard method to quantitate hepatic microsomal function [2, 3, 15].

MATERIALS AND METHODS

Chemicals. Dextromethorphan hydrobromide, dextrorphan tartrate, 3-methoxymorphinan hydrochloride, 3-hydroxymorphinan and levallorphan tartrate were kindly provided by Hoffmann-La Roche, Basel, Switzerland. Glucose-6-phosphate dehydrogenase (grade II) and NADP were from Boehringer (Mannheim, F.R.G.). Methanol and acetonitrile for HPLC were from Rathburn (Walkerburn, U.K.). All other chemicals were of analytical grade obtained from different commercial sources.

Animals. Male Sprague–Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, F.R.G.) were kept on a 12 hr light–dark cycle in temperature- and humidity-controlled quarters. They had free access to water and were fed a standard rat diet (Kliba, Basel, Switzerland). Biliary cirrhosis was induced by ligation and excision of the bile duct [16]; sham-operated animals served as controls. This group was studied 5 weeks after surgery. Micronodular cirrhosis was induced by chronic exposure to phenobarbital and carbon tetrachloride for 12 weeks according to the method of McLean *et al.* [17] as described from our laboratories [18]. Treatment was stopped 2 weeks prior to the experiments to eliminate acute effects of carbon tetrachloride and phenobarbital [2, 18]; untreated animals served as controls. Since there was no difference between the sham-operated animals and the untreated controls, these two groups were pooled and will be reported as control group.

One week prior to the study, animals were characterized by the aminopyrine breath test as previously described from our laboratory [9]. Briefly, a tracer dose of 1 μCi of [^{14}C]aminopyrine in 0.5 mL of physiological saline was administered intraperitoneally. Exhaled $^{14}CO_2$ was collected in 10 min periods for 2 hr. The results are reported as ABT_{AUC}, the area under the curve between 0 and 120 min. Total serum bile acid levels were estimated by radioimmunoassay (Becton-Dickinson, Orangeburg, U.S.A.). ALAT levels in serum were measured using a Cobas Bio centrifugal analyser (Roche, Basel, Switzerland).

In vivo dextromethorphan kinetics were performed in animals anesthetized with sodium pentobarbital 50 mg/kg body wt i.p. The left femoral artery and vein were cannulated with Portex PP 50 polyethylene tubing (Portex, Hythe, U.K.). Arterial blood pressure and rectal temperature were monitored throughout the experiment. 2.0 mg/kg dextromethorphan (calculated as free base) were injected i.v. Arterial blood samples (250 μL) were taken at 5, 10, 15, 20, 30, 50, 70, 90, 120, 150 and 180 min and immediately replaced by an equal volume of heparinized blood previously taken from a donor rat by exsanguination. After collecting the last blood sample, portosystemic shunting was assessed by a microsphere technique using ^{57}Co-labeled microspheres of 15 μm diameter [19].

Immediately thereafter, liver and spleen were removed and weighed. Microsomal fractions were prepared on the same day by differential centrifugation as previously described [2]. Cytochrome P450 content was determined by differential spectrophotometry [20]. Protein concentration was quantified [21] using serum bovine albumin as standard. Microsomal incubations of dextromethorphan were performed at 37° for 20 min and dextromethorphan and its three major metabolites analysed by a HPLC method [14].

Calculations. Disappearance of dextromethorphan from whole blood was fitted to a biexponential equation using nonlinear regression analysis as

$$y(t) = A.e^{-k_1 t} + B.e^{-k_2 t}. \quad (1)$$

Dextromethorphan clearance (Cl) was calculated as:

$$Cl = \frac{\text{Dose}}{\frac{A + B}{k_1 + k_2}}. \quad (2)$$

Apparent volume of distribution (V_D) was calculated as Cl/k_2.

The demethylaton reactions *in vitro* could adequately be described by Michaelis–Menten kinetics; K_m and V_{max} were estimated by nonlinear

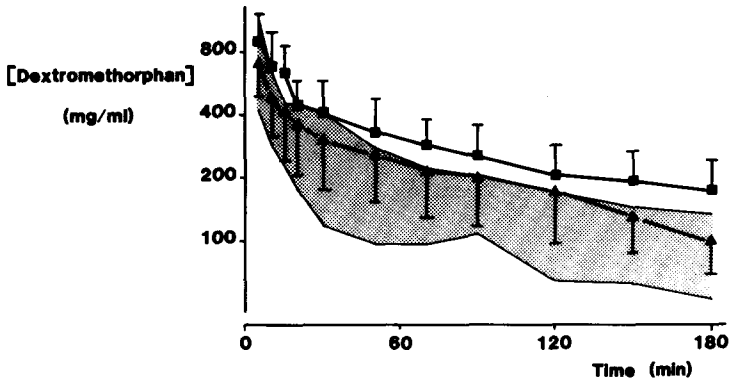

Fig. 2. Disappearance of dextromethorphan from whole blood in biliary (■ N = 5) and micronodular cirrhosis (▲ N = 7) after i.v. administration of dextromethorphan hydrobromide (2 mg/kg calculated as free base). Controls (N = 11) are indicated as the shaded area; $\bar{x} \pm$ SD are given.

regression analysis [22]. Intrinsic clearance (Cl_{int}) was calculated for the whole liver as

$$Cl_{int} = \frac{V_{max}}{K_m}. \quad (3)$$

All values were expressed as mean ± 1 standard deviation. Group means were compared by analysis of variance, followed by Student's *t*-test with the Bonferroni correction if the former showed significance. Linear regression analysis was performed by the method of least squares [23]. A probability value of < 0.05 was considered statistically significant.

RESULTS

In both experimental groups, cirrhosis was confirmed macro- and microscopically as well as by clinical findings (Table 1). As an expression of the presence of portal hypertension, spleen weights were increased in both experimental groups. Serum bile acids and ALAT levels were elevated in both cirrhotic models, but significantly more so in bile duct ligated rats. Ascites varied considerably in the cirrhotic animals (biliary: 4–26 mL, micronodular 0–47 mL). Porto-systemic shunting ranged from 0.5 to 16.4% in biliary and from 0.1 to 9.4% in micronodular cirrhosis (n.s.). ABT_{AUC} was significantly decreased to a similar extent in both cirrhotic groups.

Disappearance of dextrometrophan (2 mg/kg body wt i.v.) from whole blood is shown in Fig. 2. Dextromethorphan clearance in biliary cirrhosis (25.4 ± 5.3 mL/min/kg) was significantly lower than in both, control (62.2 ± 16.2 mL/min/kg, $P < 0.001$) and micronodular cirrhosis (48.6 ± 15.6 mL/min/kg, $P < 0.01$). Clearance in the latter did not differ significantly from the control group. The terminal elimination rate constant and volume of distribution were similar in the three groups (Table 2).

Cytochrome P450 content per mg of microsomal protein was decreased in both experimental groups, but significantly more so in the bile duct ligated as compared to the CCl_4-cirrhotic rats. Calculated per liver, however, the CCl_4-group did not differ statistically from controls, whereas the cytochrome P450 content in biliary cirrhosis was reduced to 22% of controls (Table 3). Earlier studies from our laboratories had documented that microsomal preparations from the different cirrhosis models were of similar quality as judged by protein recovery and relative specific activity of microsomal marker enzymes [2, 9].

All four demethylation reactions *in vitro* were significantly reduced in both cirrhotic models as compared to controls (Table 4). This reduction was much more pronounced in the bile duct ligated than in the CCl_4-cirrhotic animals (Table 4): In biliary cirrhosis V_{max} was only 1 to 2% of controls, the corresponding intrinsic clearance amounting to 1.3 to 7.1% of the control group. In the CCl_4 group, V_{max} and Cl_{int} were significantly higher than in biliary cirrhosis, with values ranging between 22 and 45% of controls. The four different demethylation reactions studied were affected to a similar extent within each cirrhotic group. This was further evidenced by highly significant correlations between the O- and N-demethylation reactions with *r*-values for V_{max} ranging between 0.85 and 0.96 and for Cl_{int} between 0.76 and 0.95.

K_m in control rats averaged 36 ± 11, 434 ± 119, 263 ± 72 and 12 ± 3 mM for the O-demethylations of dextromethorphan and dextrorphan and the N-demethylations of dextromethorphan and hydroxymorphinan, respectively. Substrate affinity tended to increase in BDL cirrhosis. With the exception of the demethylation of dextrorphan to hydroxymorphinan, K_m in the bile duct ligated group was reduced by about 60%. By contrast, substrate affinity in the CCl_4-cirrhotic rats remained unchanged with the exception of the metabolism of dextromethorphan to dextrorphan where K_m showed a 37% reduction.

Total intrinsic clearance (the sum of the four demethylation reactions; see last column in Table 4) correlated with dextromethorphan clearance *in vivo* (Fig. 3). Within the individual groups there were no significant correlations. As one might anticipate from the differential effect on aminopyrine (Table 1)

Table 2. Parameters of dextromethorphan pharmacokinetics *in vivo* in control (CTR), biliary cirrhosis (BDL) and micronodular cirrhosis (CCL); $\bar{x} \pm$ SD are given

	BDL (N = 5)	CCL (N = 7)	CTR (N = 11)
Clearance (mL/min/kg)	25.4 ± 5.3*†	48.6 ± 15.6	62.2 ± 16.2*†
k_{el} (hr^{-1})	0.29 ± 0.06	0.38 ± 0.06	0.37 ± 0.11
V_D (L/kg)	5.3 ± 1.2	7.8 ± 1.5	11.7 ± 7.1

* $P < 0.001$ BDL vs CTR; † $P < 0.01$ BDL vs CCL.

Table 3. Cytochrome P450 content in microsomes prepared from bile duct ligated rats (BDL), CCL_4/phenobarbital (CCL) and controls (CTR); $\bar{x} \pm$ SD are shown

	BDL (N = 5)	CCL (N = 7)	CTR (N = 11)
Cytochrome P450 (nmol/mg protein)	0.26 ± 0.07	0.61 ± 0.18	0.76 ± 0.10*†‡
Cytochrome P450 (nmol/liver)	62.2 ± 32.7	208 ± 117.7	287.8 ± 66.7*§

* $P < 0.001$ BDL vs CTR; † $P < 0.002$ BDL vs CCL; ‡ $P < 0.05$ CCL vs CTR; § $P < 0.05$ BDL vs CCL.

Table 4. Maximal velocities (V_{max}) nmol/min, calculated for total microsomal fractions of dextromethorphan O- and N-demethylations *in vitro* and total intrinsic clearance (Cl_{int}). Cl_{int}: Sum of all O- and N-demethylations calculated as V_{max}/K_m per total microsomal fraction (mL/min)

	O-Demethylation		N-Demethylation		
	DEM-DOR V_{max}	DOR-HOM V_{max}	DEM-MEM V_{max}	MEM-HOM V_{max}	Total Cl_{int}
CTR $\bar{x}$	174	169	526	140	18.6
SD	73	91	260	57	6.2
BDL $\bar{x}$	4	2	6	2	0.9
SD	4	2	5	2	0.9
CCL $\bar{x}$	54	62	152	51	6.9
SD	45	55	150	42	5.8
P BDL vs CTR	0.001	0.001	0.001	0.001	0.001
P CCL vs CTR	0.001	0.013	0.003	0.003	0.001
P CCL vs BDL	0.03	0.036	0.05	0.028	0.05

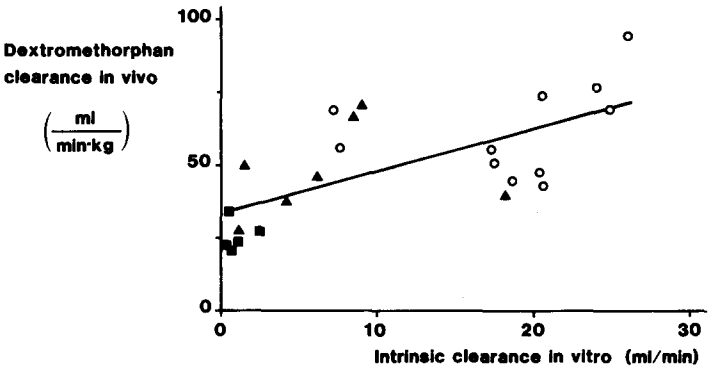

Fig. 3. Relationship between clearance of dextromethorphan *in vivo*, *in vitro* in control rats (○) and rats with biliary (■) or micronodular (▲) cirrhosis. The regression equation is $y = 1.493x + 33.3$ ($r = 0.681$, $P < 0.001$).

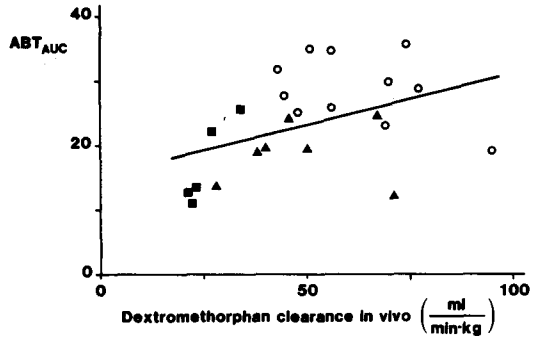

Fig. 4. Relationship between aminopyrine N-demethylation *in vivo* (ABT_{AUC}), and clearance of dextromethorphan *in vivo* in control rats (○), in biliary (■) and in micronodular cirrhosis (▲). The regression equation is $y = 0.159x + 15.32$ ($r = 0.424$, $P < 0.05$).

and dextromethorphane clearance (Table 2), the relationship between these two ABT_{AUC} and dextromethorphan clearances *in vivo* was much weaker (Fig. 4).

DISCUSSION

Our results demonstrate a differential effect of two models of liver cirrhosis in the rat on the *in vivo* handling of the two test compounds aminopyrine and dextromethorphan: while *in vivo* N-demethylation of aminopyrine was reduced to a similar extent in both models, dextromethorphan clearance *in vivo* was reduced in biliary but not in micronodular cirrhosis. These effects could be partially explained by alterations in the different N- and O-demethylation reactions governing dextromethorphan elimination. Although the correlation between clearance *in vitro* and *in vivo* was good, it explained only 46% of the variance, clearly demonstrating that other factors, not assessed in the present study, contribute to reduced drug clearance in cirrhosis.

Dextromethorphan is a highly lipophilic compound with an octanol/water coefficient of 40 and a blood/plasma ratio of 1.5 (own unpublished results). In this respect, the disposition of dextromethorphan resembles the pharmacokinetics of propranolol in the rat [24, 25]. It may be speculated, therefore, that hepatic uptake and distribution, as well as redistribution are important determinants of hepatic dextromethorphan pharmacokinetics. Accordingly, the *in vivo* clearance of dextromethorphan in our control rats was similar to reported liver blood flow in rats [26]. As a high extraction compound, i.v. clearance of dextromethorphan, therefore, should primarily be determined by hepatic blood flow and to a lesser extent by its intrinsic clearance [27]. The close correlations between *in vivo* clearance and intrinsic clearance *in vitro* seem to contradict this hypothesis. It has to be kept in mind, however, that intrinsic clearance could explain only 48% of the variability of clearance *in vivo* ($r = 0.68$). The drastic 95% reduction of intrinsic clearance in biliary cirrhosis is in contrast to the relatively modest decrease of *in vivo* clearance of 59%.

A reduction in intrinsic clearance has been identified as a major determinant of decreased drug metabolism in liver cirrhosis [7, 8]. Our study agrees with this view as exemplified by the correlation shown in Fig. 3. For aminopyrine N-demethylation we have recently demonstrated that intrinsic clearance per volume of hepatocyte is maintained in micronodular cirrhosis [2], thus supporting the "intact cell hypothesis" [31] for this model. Although in the present study no morphometric measurements were performed, the drastic reduction in intrinsic clearance in the biliary cirrhosis to 5% of controls advocates that the "sick cell hypothesis" applies to this model, particularly since morphometrically determined hepatocellular mass is maintained in biliary cirrhosis with similar functional impairment as described in the present study [9]. Different isoenzymes seem to be involved in dextromethorphan demethylation, among them, ethylmorphine-*N*-demethylase (EC 1.14.14, [28]), and P450db1 [29]. Cirrhosis may cause selective impairment of different oxidative pathways [1, 6, 30]: While some isoenzymes may be unaltered, others are impaired. This has been demonstrated for androgen hydroxylation in a model of micronodular cirrhosis and a choline deficient cirrhotic model [32, 33]. The excellent correlations between the four different reactions measured in our assay, however, suggest a strong similarity of the isoenzymes involved in dextromethorphan demethylation. Aminopyrine N-demethylation is mediated by a variety of different P450 isoenzyme [34] which seem to be affected less by cirrhosis.

The differential effects of CCl_4 and biliary cirrhosis have been noted before [4]. Part of this could be due to retention of compounds normally excreted into bile in biliary cirrhosis; thus, bile acids have been shown to alter cytochrome P450-related functions [35]. Another factor could be shunting which has been shown to selectively affect male specific cytochrome P450 isoenzymes [36].

Our data re-emphasize the facts that (a) drug metabolising isoenzymes in cirrhosis can be affected differentially in cirrhosis [5, 30, 32, 33, 36] and by different models of cirrhosis [4] and (b) that factors other than the so-called intrinsic clearance [27] affect drug metabolism. These include hemodynamic alterations such as shunting [36] and alterations of microvascular exchange [3] as well as retention of compounds normally excreted into bile in the case of models including a cholestatic component such as bile duct ligation-induced biliary cirrhosis [35]. These findings demonstrate that the "intact cell" vs "sick cell" hypothesis [31] are applicable at best to partial functions, only.

In conclusion, it has been demonstrated that *in vivo* disposition of dextromethorphan is impaired in rats rendered cirrhotic by bile duct ligation, but not by chronic exposure to CCl_4, whereas the aminopyrine breath test is impaired to the same degree in both models. Dextromethorphan, therefore, appears to measure different aspects of microsomal liver function than aminopyrine. In addition to its use to determine hydroxylator

phenotype [13, 14], this compound might be a useful tool to assess microsomal liver function in liver disease.

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