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HEPATIC EXTRACTION OF CHENODEOXYCHOLIC ACID IN DOGS CHRONICALLY INTOXICATED WITH DIMETHYLNITROSAMINE*

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Abstract—The pharmacokinetics of chenodeoxycholic acid (CDCA) in hepatic dysfunction were evaluated by analyzing the plasma disappearance curves after simultaneous administration of [^{3}H]- and [^{14}C]-CDCA through the femoral and portal veins, respectively, in dogs chronically intoxicated with dimethylnitrosamine (DMN). The plasma concentration–time curve of intravenously administered [^{3}H]-CDCA was best fitted to a three-exponential equation, while that of intraportally administered [^{14}C]-CDCA was fitted to either a two- or a three-exponential equation. In the DMN-intoxicated dogs, significant decreases were observed in total body plasma clearance (CL_p), hepatic extraction ratio (E_H) and apparent intrinsic clearance (CL_{int}) compared to those of the untreated (control) dogs. The hepatic blood flow (Q_H), calculated from CL_p, CL_{int} and blood-to-plasma concentration ratio (R_B) according to the equation reported by Wilkinson and Shand [*Clin. Pharmac. Ther.* **18**, 377 (1975)], was reduced to approximately 70% in the DMN-intoxicated dogs compared to the control dogs. The bindings of CDCA to plasma and liver cytosol fraction were determined by equilibrium dialysis; no significant difference was observed in the unbound fraction between the DMN-treated and control dogs. By comparing both pharmacokinetic parameters obtained from intravenous and intraportal administration, the usefulness of the oral bile acid tolerance test was examined. From these findings, it was suggested that the decrease in the CL_p of the DMN-intoxicated dogs was due to both the decrease in Q_H and that in CL_{int}, and that the decrease in CL_{int} may be due not to an alteration of plasma or cytosol binding but to that of a carrier-mediated transport system. It is also suggested that the measurement of fasting plasma bile acid concentration or the oral bile acid tolerance test is more sensitive for the detection of hepatic dysfunction than the intravenous bile acid tolerance test.

A canine model of hepatic cirrhosis created by intermittent oral administration of dimethylnitrosamine (DMN), which was first proposed by Madden *et al.* [1], has the advantage of producing hepatic cirrhosis which is stable or progressive for at least 5 months after discontinuing DMN administration. Previously we examined the hepatic transport of indocyanine green (ICG) in dogs using this model and suggested its usefulness as a model for chronic hepatic dysfunction.¶

Recent development of a pharmacokinetic model of the clearance concept [2–5] has made it possible to predict clearly the effects of alterations in physiological factors such as blood flow, drug-metabolizing activity and protein binding. Many investigations concerning fasting or postprandial serum bile acid concentration and the bile acid tolerance test after oral or intravenous administration in liver disease have been made since a methodological development in the assay of bile acids [6–8]. Hepatic blood flow (Q_H) [3], carrier-mediated hepatic uptake [9–11], plasma protein binding [12, 13], liver cytosol binding [14, 15], and biliary excretion [16] have been suggested as the determining factors in the hepatic clearance (CL_H) of bile acids.

The present study was designed to evaluate separately the effects of Q_H and hepatic intrinsic clearance (CL_{int}) as the determining factors in liver dysfunction by comparing pharmacokinetic parameters of chenodeoxycholic acid (CDCA) after simultaneous administration of [^{3}H]- and [^{14}C]-CDCA via intravenous and intraportal routes, respectively, in dogs chronically intoxicated with DMN. The bindings of CDCA to plasma protein and liver cytosol fraction were also examined *in vitro*. In accordance with these pharmacokinetic findings, the clinical significance of fasting or the level of postprandial serum bile acids and the bile acid tolerance test after oral or intravenous administration are discussed.

MATERIALS AND METHODS

Animals and their preparation. Adult mongrel dogs (8–14 kg) were used. DMN intoxication was produced according to the method of Madden *et al.* [1] after slight modification. Briefly, under sodium pentobarbital anesthesia (25 mg/kg, i.v.), a laparotomy was performed through a midline incision,

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a polyethylene catheter (i.d. 1.0 mm; length 70 cm) was introduced into the portal vein through a branch of the superior mesenteric vein, and the other end of the catheter was fixed to the skin of the neck. One week after the operation, DMN was given intraportally at a dose of 2 mg/kg without anesthesia once a week for 6 weeks. Treated animals were used as the intoxicated dogs 4–6 weeks after the final dose of DMN. Healthy dogs without any treatment were used as the control dogs.

Materials. Dimethylnitrosamine was purchased from the Wako Pure Chemicals Co. Ltd., Tokyo, Japan. [^{14}C]- (50.0 mCi/mmole) and [^{3}H]-chenodeoxycholic acids (38.6 Ci/mmole) were purchased from the New England Nuclear Corp., Boston, MA, U.S.A. and found to be more than 98% pure by thin-layer chromatography. Sodium pentobarbital was obtained from the Abbott Laboratories, North Chicago, IL.

Blood and bile samplings. After overnight fasting, under sodium pentobarbital anesthesia (25 mg/kg, i.v.), the femoral artery and vein were cannulated with polyethylene catheters (i.d. 1.9 mm; length 30 cm for the artery, and i.d. 1.0 mm, length 70 cm for the vein). Blood samples were taken from the femoral artery catheter for assay of the total plasma bile acid concentration. After the laparotomy, the cystic duct was ligated and the common bile duct was cannulated with a polyethylene catheter (i.d. 1.0 mm; length 90 cm) for bile sampling. In the control dogs a polyethylene catheter (i.d. 1.0 mm; length 70 cm) was introduced into the portal vein through a branch of the superior mesenteric vein. In the intoxicated dogs, the portal catheter previously inserted for DMN administration was used.

Tracer amounts of [^{14}C]-CDCA (3.5 to 8.0 μCi) and [^{3}H]-CDCA (10.5 to 23.0 μCi) were dissolved in 2 ml of saline and were administered simultaneously, the former through the portal vein catheter and the latter through the femoral vein catheter. After the catheters were flushed with saline, blood samples were obtained through the femoral artery catheter at 1, 1.5, 2, 3, 4, 5, 7, 10, 15, 20, 25, 30, 40, 60, 75, 90 and 120 min. Bile was collected at 5-min intervals for the first half hour, 10-min intervals for the second half hour, and 15-min intervals from 60 to 150 min. Physiological saline was infused at about 100 ml/hr through the right external jugular cannula. At the end of the experiments, whole or part (approximately 10 g) of the liver specimen was removed and stored at −40°.

Analytical methods. For the blood samples, the plasma concentration of [^{3}H]- and/or [^{14}C]-CDCA was determined in a Packard Tri-Carb counter after an aliquot (0.5 ml) was placed in a scintillation vial containing 10 ml of liquid scintillation solution (0.1 g of POPOP*, 4.0 g of PPO, and 500 ml of Triton X-100/liter of toluene). Bile volume was measured gravimetrically, assuming a density of 1.0 for bile. The radioactivity of ^{3}H and/or ^{14}C was assayed as described above using 5 μl of bile. The concentrations of the total plasma bile acids were assayed in each dog by the enzymatic and fluorometric method [17].

Pharmacokinetic analysis. Plasma disappearance curves were fitted to the following equation with a non-linear least squares method [18], using a HITACH M-200/280H digital computer:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + (Ce^{-\gamma t}) \quad (1)$$

The plasma concentration of [^{3}H]-CDCA after intravenous administration followed a three-exponential equation, while that of [^{14}C]-CDCA after intraportal administration followed a two- or a three-exponential equation, where the best fit number of exponentials was judged by the residual sum of squares and AIC (Method 1). Since in linear pharmacokinetics the rate constants, α, β and γ, should be constant and independent of the route of administration [19], the values of A, B and C for [^{3}H]-CDCA were also calculated fixing the three rate constants (α, β and γ) calculated from the disappearance curve of [^{14}C]-CDCA (Method 2). The total body plasma clearance (CL_p) and the apparent hepatic intrinsic clearance (CL_{int}) were calculated by the following equations respectively:

$$CL_p = \frac{\text{Dose}[^3\text{H}]}{\text{AUC}_p[^3\text{H}]} \quad (2)$$

$$CL_{int} = \frac{\text{Dose}[^{14}\text{C}]}{\text{AUC}_p[^{14}\text{C}]} \quad (3)$$

where AUC[^{3}H] and AUC[^{14}C] were calculated by the following equation [20]:

$$\text{AUC}_p = \frac{A}{\alpha} + \frac{B}{\beta} + \left(\frac{C}{\gamma}\right) \quad (4)$$

The hepatic extraction ratio (E) was calculated by the following equation [21]:

$$E_H = 1 - \frac{CL_p}{CL_{int}} \quad (5)$$

And the hepatic blood flow (Q_H) was calculated by the following equation [22]:

$$Q_H = \frac{1}{R_B} \cdot \frac{CL_{int} \cdot CL_p}{CL_{int} - CL_p} \quad (6)$$

where R_B is the blood-to-plasma concentration ratio, which was determined *in vitro* using the bloods obtained from the control and intoxicated dogs.

In the above described pharmacokinetic analysis based on the data after intravenous and portal administration of [^{3}H]- and [^{14}C]-CDCA, respectively, we assumed that linear pharmacokinetics apply and that the intravenously and portally administered CDCA are cleared from the circulation in an identical manner.

The initial volume of distribution (V_1) after intravenous administration of [^{3}H]-CDCA was calculated by the following equation:

$$V_1 = \frac{\text{Dose}[^3\text{H}]}{C(0)} = \frac{\text{Dose}[^3\text{H}]}{A + B + C} \quad (7)$$

where $C(0)$ is the plasma concentration of [^{3}H]-CDCA at time 0.

Blood-to-plasma concentration ratio (R_B). After

* POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)] benzene; and PPO = 2,5-diphenyloxazole.

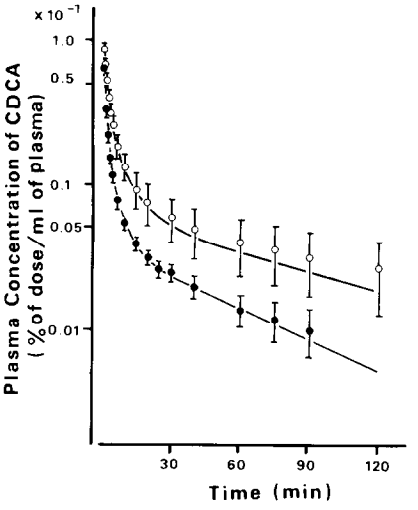

Fig. 1. Plasma disappearance of [^{3}H]-chenodeoxycholic acid (CDCA) after intravenous administration of a tracer dose. Each point represents the mean ± S.E. of four dogs. Curves were calculated by a least squares method [18] using a digital computer. Key: (●) control; and (○) intoxication.

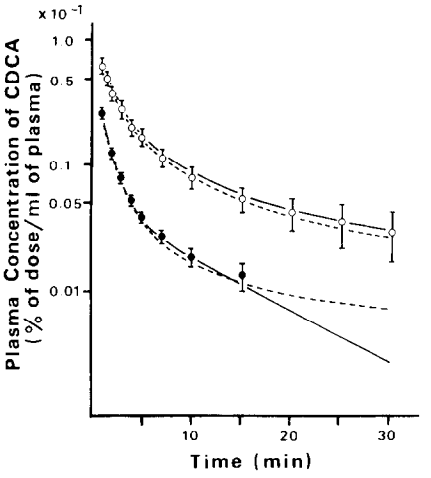

Fig. 2. Plasma disappearance of [^{14}C]-chenodeoxycholic acid (CDCA) after intraportal administration of a tracer dose. Each point represents the mean ± S.E. of four dogs. Solid curves were calculated according to Method 1 by a least squares method [18] using a digital computer, while broken curves were calculated according to Method 2 (see Materials and Methods). Key: (●) control; and (○) intoxication.

preincubation of 5-ml heparinized whole blood samples obtained from the control and intoxicated dogs at 37° for 5 min, a tracer amount of [^{14}C]-CDCA (100 μl) was added and then the radioactivities of ^{14}C in the whole blood and the plasma separated by centrifugation were assayed at 20 sec, and at 15, 30 and 50 min after addition of [^{14}C]-CDCA.

Binding of CDCA to plasma protein and liver cytosol. In each dog except No. 1, the liver homogenate (33%, w/v) was prepared in 0.13 M Sörensen buffer (pH 7.4), and a 100,000 *g* supernatant fraction was obtained as previously reported [23]. The bindings of CDCA to plasma and liver supernatant fraction were determined by equilibrium dialysis at 37° for 14 hr in the dialysis cell using a semipermeable membrane (Spectrapor; Spectrum Medical Industries Inc., Los Angeles, CA, U.S.A.). After dialysis, the radioactivities of ^{14}C in both the plasma or supernatant fraction and the buffer were assayed. The unbound fraction of CDCA was calculated by the following equation:

$$\text{Unbound fraction (\%)} = \frac{^{14}\text{C in the buffer side}}{^{14}\text{C in the protein side}} \times 100 \quad (8)$$

Statistical analysis. All means are presented with the standard error. Student's *t*-test was utilized to determine a significant difference between the control and intoxicated groups with $P = 0.05$ as the minimal level of significance.

RESULTS

Analysis of plasma disappearance curves. The plasma concentration–time curves after intravenously administered [^{3}H]-CDCA and intraportally administered [^{14}C]-CDCA are shown in Figs. 1 and 2 respectively. A marked difference was shown between the control and intoxicated dogs after intraportal administration. The plasma concentration–time curves after intravenous administration of [^{3}H]-CDCA were best fitted to a three-exponential equation in all dogs studied, while those after intraportal administration of [^{14}C]-CDCA were fitted to either a two- or a three-exponential equation (Method 1). The values of A, B and C were further calculated by fitting to a three-exponential equation using the fixed values of α, β and γ which were obtained from the analysis of [^{3}H]-CDCA disappearance curves (Method 2). The calculated pharmacokinetic parameters are summarized in Table 1. The values of CL_{int}, E_H and Q_H were calculated by both methods. In the intoxicated dogs, significant decreases were observed in CL_p, E_H and CL_{int} calculated by either Method 1 or Method 2. The CL_p, E_H and CL_{int} values of the intoxicated dogs were approximately 45, 60 and 25% of those of the control dogs respectively. The Q_H calculated from these parameters in the intoxicated dogs was approximately 70% of that of the control dogs, although the difference was not statistically significant probably due to an anomaly of dog 14. Without dog 14, the mean Q_H of 24.0 ± 8.1 ml/min/kg was obtained, and it is significantly different from the control dogs ($P < 0.05$).

Total plasma bile acids concentration. Figure 3 shows the reciprocal of CL_{int} versus the total plasma bile acids concentration except for dog No. 1. A marked increase in the total plasma bile acids concentration was observed in the intoxicated dogs.

Biliary excretion. In dogs, CDCA was excreted into bile after conjugation with taurine in the liver cell. In the present study, the amounts of ^{14}C excreted for 150 min after intraportal administration were 72.4 ± 18.0% in the control dogs and 75.3 ± 13.0% in the intoxicated dogs, and those of ^{3}H were 53.5 ± 13.6% in the control dogs and 54.8 ± 8.9% in the intoxicated dogs. No significant difference was observed in the amount of either ^{14}C or ^{3}H between

Table 1. Pharmacokinetic parameters of CDCA

	V_1 (ml/kg)	CL_p (ml/min/kg)	Method I* CL_{int} (ml/min/kg)	Method I* E_H	Method I* Q_H (ml/min/kg)	Method II* CL_{int} (ml/min/kg)	Method II* E_H	Method II* Q_H (ml/min/kg)
Control								
1	55.2	18.2	106.8	0.830	42.1	63.5	0.713	49.0
2	62.4	22.7	78.5	0.711	61.2	62.8	0.639	68.0
3	169.7	16.7	83.9	0.801	40.0	53.1	0.685	46.8
4	106.2	25.4	75.4	0.663	73.5	57.8	0.560	87.1
Mean	98.4	20.8	86.2	0.751	54.2	59.3	0.649	62.7
S.E.	26.3	2.0	7.1	0.039	8.0	2.4	0.033	9.4
Intoxication								
11	82.2	3.3	7.3	0.546	9.5	6.2	0.466	11.1
12	96.7	8.1	16.4	0.505	25.2	15.0	0.458	27.7
13	64.5	14.7	38.6	0.618	37.4	31.8	0.537	43.1
14	44.9	11.2	14.8	0.244	72.5	13.8	0.188	93.6
Mean	72.1	9.3†	19.3‡	0.478§	36.2	16.7‡	0.412§	43.9
S.E.	11.2	2.4	6.7	0.082	13.4	5.4	0.077	17.8

* See text for details.
†–§ Significantly different from the control at P = †0.02, ‡0.01 and §0.05 respectively.

the two groups. We did not determine the conjugate percent of the radioactivity in the bile. Therefore, further pharmacokinetic analysis on biliary excretion was not performed.

Blood-to-plasma concentration ratio (R_B). In both the control and intoxicated dogs, the values of R_B at 20 sec, 15, 30 and 50 min after administration of [^{14}C]-CDCA remained nearly constant, namely 0.521 (the mean value) in the control dogs and 0.635 (the mean value) in the intoxicated dogs. The red blood cell-to-plasma concentration ratios calculated using these R_B values and the hematocrit values (0.526 in the control dogs and 0.395 in the intoxicated dogs) were 0.09 in the control dogs and 0.08 in the intoxicated dogs, suggesting that only a fraction of CDCA distributed into red blood cells.

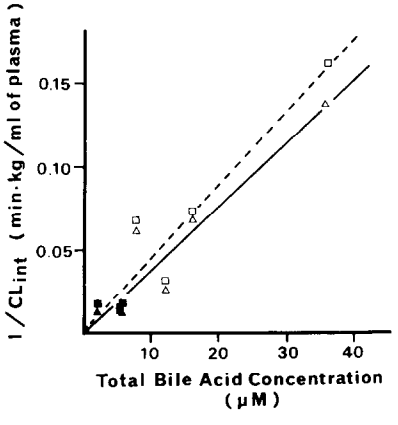

Fig. 3. Relationship between the reciprocal of hepatic intrinsic clearance (CL_{int}) of chenodeoxycholic acid (CDCA) and total bile acid concentration in plasma. A solid line was calculated from the data for Method 1 by a least squares method [18] using a digital computer, while a broken line was calculated from those for Method 2. Key: (▲) control (Method 1), (■) control (Method 2), (△) intoxication (Method 1), and (□) intoxication (Method 2).

Binding of CDCA to plasma protein and liver cytosol. The unbound fractions of CDCA in plasma and liver cytosol determined by equilibrium dialysis are listed in Table 2. No significant difference was observed in the unbound fractions between the control and intoxicated dogs.

DISCUSSION

The present study was designed to examine the hepatic extraction of double-labeled chenodeoxycholic acids ([^{14}C]- and [^{3}H]-CDCA) after simultaneous administration to dogs without enterohepatic circulation of bile acids. This method made it possible to determine the CL_p, CL_{int}, E_H and Q_B for CDCA of individual animals by pharmacokinetic analysis. These pharmacokinetic parameters were

Table 2. Free fractions of CDCA in plasma and liver cytosol*

	Dog	Unbound CDCA(%) Plasma	Liver cytosol
Control†	2	1.86	24.6
	3	2.48	23.1
	4	1.72	20.2
	Mean	2.02	22.6
	S.E.	0.24	1.3
Intoxication	11	2.77	23.6
	12	1.51	17.9
	13	2.37	19.4
	14	1.54	18.1
	Mean	2.05	19.8
	S.E.	0.31	1.3

* Binding was determined by the equilibrium dialysis method at 37° (see text).
† Dog No. 1 was not determined.

compared between the control and chronically intoxicated dogs with DMN. In the present analysis, we assumed that the total radioactivity in plasma is directly indicative of CDCA itself. The analysis of radioactive species was not performed in the present study; however, this assumption may be reasonable, since in humans the AUC as determined by gas–liquid chromatographic analysis to measure the concentration of unconjugated CDCA in blood was almost the same as that determined from the blood radioactivity after injection of [^{3}H]-CDCA [24]. Most of the major bile acids are highly extracted by the liver, although the conjugation and the number and position of hydroxy groups affect E_H [25, 26]. E_H of CDCA was reported previously to be 0.624 for normal humans [24] and 0.538 for intact rats [26]. In the present study, high extraction ratios of CDCA were also shown in the control dogs, i.e. 0.751 by Method 1 and 0.649 by Method 2 (Table 1). The values of Q_H in the control dogs (54.2 ± 8.0 ml/min/kg for Method 1 and 62.7 ± 9.4 for Method 2) were comparable to that reported by Huet *et al.* (48.6 ± 2.9 ml/min/kg) [27]. According to the well-stirred model [4, 21], CL_H is described by the following equation:

$$CL_H = Q_H \cdot E_H = \frac{Q_H \cdot CL_{int}/R_B}{Q_H + CL_{int}/R_B} \quad (9)$$

and, since the elimination of CDCA is mainly by hepatic extraction, the total blood clearance (CL_{tot}) nearly equals to CL_H:

$$CL_{tot} \simeq CL_H \quad (10)$$

As can be seen in equations 9 and 10, either the decrease in Q_H or that in CL_{int} can affect the decrease in CL_{tot}. Therefore, the decrease in CL_{tot} which was observed in the intoxicated dogs may be due to the decreases in both Q_H and CL_{int}, though the decrease in CL_{int} was greater than that in Q_H (Table 1). In addition, a significant decrease in E_H may be explained by a marked decrease in CL_{int}.

As factors affecting CL_{int} of CDCA, binding to plasma albumin [12, 13] and liver cytosol [14, 15], carrier-mediated transport in plasma membrane [9–11, 16] and metabolic conjugation could be considered. Since conjugated bile acids have been known to be highly extracted by the liver as well as unconjugated bile acids [25, 26], metabolic conjugation as a determining factor might be excluded. In addition, no significant difference was observed in the binding of CDCA to either plasma protein or liver cytosol (Table 2). Therefore, the decrease in the carrier-mediated transport ability of plasma membrane was suggested as the cause of the remarkable decrease in CL_{int} of CDCA.

In relation to these findings concerning alterations of pharmacokinetic parameters in the hepatic dysfunction model, we now discuss the quantitative evaluation of fasting or postprandial serum bile acid concentrations, and the bile acid tolerance test for the liver after oral or intravenous administration of bile acids in humans. In fasting, the peripheral venous concentration of CDCA is constant [28], and this suggested that the absorption rate of CDCA by the small intestine is equal to the hepatic uptake rate. Since under the physiological concentration of bile acids in the portal vein the hepatic uptake of bile acids can be assumed to follow first-order kinetics [29], CL_H of CDCA does not depend on either portal venous or hepatic arterial concentration of CDCA. Thus, the peripheral venous concentration of CDCA is given by the following equation [21]:

$$C = \frac{I}{CL_{int}} \quad (11)$$

where C is the peripheral venous concentration of CDCA and I is the intestinal absorption rate. According to equation 11, in the fasting condition the peripheral concentration of CDCA is reciprocally proportional to CL_{int} if I is kept constant in normal subjects and patients with hepatic dysfunction. In Fig. 3 the relationship between CL_{int} of CDCA and total plasma bile acids concentrations in the control and DMN-intoxicated dogs was plotted on the assumption that CL_{int} of CDCA can reflect that of the total bile acids. A comparatively good correlation was obtained both in the control and intoxicated dogs. Slight deviations from the regression line may be due to interindividual differences in the concentration ratio of CDCA to total plasma bile acids or in the intestinal absorption rate. This result suggested that the fasting bile acid concentration reflects the value of CL_{int} and, therefore, could be a good index of liver function. Significance of the oral bile acid tolerance test and the measurement of postprandial bile acid concentration in the liver function test [30–32] could be explained from the same point of view. On the other hand, in the intravenous bile acid tolerance test [33–36], the total body clearance (CL_p) includes both Q_H and CL_{int}. Furthermore, in various liver diseases the decrease in Q_H is not always accompanied by a parallel decrease in CL_{int}. In fact, Cohn *et al.* [37] reported an increase in Q_H in patients with alcoholic hepatitis. This may explain the observation that in some liver diseases the intravenous bile acid tolerance test does not show a significant alteration.

In conclusion, it is suggested that the decrease in hepatic extraction of CDCA in DMN-intoxicated dogs as a model of chronic hepatic dysfunction is due mainly to the alteration in the ability of carrier-mediated transport. It is also suggested that the measurement of fasting bile acids concentration or the oral bile acid tolerance test can detect more sensitively the hepatic dysfunction than the intravenous bile acid tolerance test does.

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