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EFFECT OF CHLORPROMAZINE ON HEPATIC TRANSPORT OF INDOCYANINE GREEN IN RATS*

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Abstract—The effect of chlorpromazine hydrochloride (CPZ) on the hepatic transport of indocyanine green (ICG) was studied in the rat, in an attempt to elucidate the mechanisms of hepatotoxicity of CPZ *in vivo*, by comparing the pharmacokinetic parameters of ICG after bolus and chronic administration of CPZ. Delays were shown in both plasma disappearance and biliary excretion of ICG in the CPZ-treated rats (10 and 15 mg/kg intraportal bolus administration). Significant decreases were observed in the pharmacokinetic parameters, V_2 and total body clearance (CL_{tot}) in CPZ 10 mg/kg treated rats and k_{34}, V_2 and CL_{tot} in CPZ 15 mg/kg treated rats, while a significant increase was observed in k_{21} in both CPZ-treated groups; V_1 was not altered. The apparent liver-to-plasma concentration ratio ($K_{p,app}$) of ICG at 50 min after i.v. administration was decreased significantly in CPZ 15 mg/kg treated rats when compared to control rats, suggesting an alteration in the distribution of ICG to the liver by CPZ. Bile flow rates decreased immediately after bolus intraportal administration of CPZ in both CPZ-treated groups, and they then returned progressively to the basal levels. The output of bile acids was also inhibited by CPZ in a time-dependent and reversible manner and the bile acid independent fraction of bile flow was decreased significantly in both CPZ-treated groups. Chronic treatment with CPZ (10 or 20 mg/kg, i.p., per day for 3 weeks) did not alter either the pharmacokinetic parameters or the bile secretion profile of ICG, although there were significant decreases in body and liver weights in CPZ-treated groups. This may have been due to the rapid metabolism and excretion of CPZ in the rat when compared to humans. It is proposed that the acute toxic effect of CPZ on hepatic transport of ICG in the rat may be due mainly to the time-dependent and reversible cholestasis induced by CPZ, and that chronic treatment with CPZ may not alter the hepatic transport of ICG in the rat.

Chlorpromazine hydrochloride (CPZ), one of the oldest major tranquillizers, is used extensively in the treatment of anxiety states and psychiatric disorders. The pathogenesis of cholestatic jaundice, which occurs in 1–2% of patients treated with CPZ, has been thought to result from hypersensitivity to CPZ [1]. Other observations suggest that the intrinsic hepatotoxicity of CPZ may contribute to hepatic injury [2]. Almost 50% of patients taking CPZ for prolonged periods of time show abnormal liver function tests, e.g. increased sulfobromophthalein (BSP) retention [2], higher values of GOT (glutamic-oxaloacetic transaminase activity) and GPT (glutamic-pyruvic transaminase activity) [3], and morphological abnormalities in liver biopsies [2], suggesting a direct hepatotoxic effect of CPZ. Furthermore, in experimental animal studies it has been reported that CPZ decreases the bile flow in the rhesus monkey [4] as well as in isolated perfused rat liver [5–7], induces enzyme leakage from rat liver slices [8] and isolated rat hepatocytes [9, 10], inhibits the respiration of isolated rat mitochondria [11] and isolated rat hepatocytes [10], and reduces the fluidity of rat liver plasma membrane [12]. Eckhardt and Plaa [13, 14] reported the increased BSP retention in mice and rats by various phenothiazine derivatives, and they suggested that the plasma BSP retention was due to a decreased hepatic blood flow [15, 16].

Indocyanine green (ICG), a synthetic dye, is widely used to assess hepatic function as measured by the plasma retention of this dye after intravenous injection. After intravenous administration, ICG is distributed in the plasma volume without extravascular distribution and is removed exclusively by the liver into the bile without biotransformation [17, 18].

The purpose of this study was to determine the effects of CPZ on the hepatic transport of ICG in rats, in an attempt to elucidate the mechanisms of hepatotoxicity of CPZ *in vivo* by comparing the pharmacokinetic parameters of ICG after bolus and chronic administration of CPZ.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (Nihon Seibutsuzairyo Co., Tokyo, Japan) weighing 270–300 g were used. CPZ chronically treated rats were produced by repeated intraperitoneal injection of CPZ at doses of 10 or 20 mg/kg per day for 3 weeks.

Materials

ICG was purchased from the Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan. CPZ was supplied by the Yoshitomi Pharm. Ind. Co. Ltd., Osaka, Japan. 3α-Hydroxysteroid dehydrogenase was purchased from the Sigma Chemical Co., St. Louis, MO. All

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other reagents were commercial products and of analytical grade.

Experimental procedure

Bolus injection study. Under light ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing for ICG administration and blood sampling respectively. The common bile duct was cannulated with PE-10 tubing. The injection of CPZ and physiological saline into the portal vein was accomplished with PE-10 tubing attached to a 27-gauge needle. The cannula was fixed with surgical glue (Aron Alpha, Sankyo Co. Ltd., Tokyo, Japan). In the first experiment, after recovery of the rectal temperature to 37° with a heat lamp, bile was sampled at 10-min intervals for 20 min, and blood (0.5 ml) was obtained in heparinized polyethylene centrifuge tubes (Beckman Instruments, Fullerton, CA) for GOT and LDH (lactate dehydrogenase activity) assay. Then, CPZ was administered at 10 or 15 mg/kg through the portal vein cannula and, 30 sec later, a 3-μmole dose of ICG (8.5 mg/kg) was administered intravenously through the femoral vein cannula. Bile was sampled at 10-min intervals for 4 hr, and blood was sampled at given times for 2 hr. The control rats were given physiological saline instead of CPZ. The rectal temperature of the rats was maintained at 37° with a heat lamp. In the second experiment, bile samples were collected for 4, 8 or 24 hr after CPZ and ICG administration as described above, and the total bile volume excreted and the total amount of ICG excreted for respective periods were determined. In the third experiment, at 50 min after CPZ and ICG administration, rats were killed by bleeding from a carotid artery, and then a 50% homogenate was prepared in Tris–HCl buffer (pH 7.4). The total amount of ICG in the liver was determined as described previously [19]. The liver-to-plasma concentration ratio (K_p) of ICG was calculated by $K_p = C_L/C_p$, where C_L is the concentration of ICG in the liver and C_p is that in plasma [20].

Chronic injection study. Twenty-four hours after the final i.p. injection of CPZ (10 or 20 mg/kg), the rats were anesthetized with ether, and the femoral vein, the artery and the common bile duct were cannulated in the same manner as described before. After recovery of the rectal temperature to 37° with a heat lamp, the study was carried out in the same manner as described for the bolus injection study except for CPZ administration.

Bile flow study. In the control rats, under a constant infusion of physiological saline at 1.06 ml/hr, bile was sampled at 1-hr intervals for 10 hr. In the CPZ-treated rats, after intraportal bolus administration of CPZ at a dose of 10 or 15 mg/kg, bile was sampled at 10-min intervals for 50 min.

Analytical methods

For ICG analysis in the blood sample, after centrifugation in a table-top microfuge (Beckman Instruments), 0.1 ml of plasma was diluted with 3 ml of distilled water and immediately measured at 800 nm in a Hitachi 356 spectrophotometer (Hitachi Koki Co. Ltd., Tokyo, Japan). For the bile sample, after 0.1 ml of bile was diluted with 4 ml of distilled water, the samples were centrifuged at 4° for 10 min at 12,000 egv (approximately 12,000 *g*) in a Hitachi 20PR-5 centrifuge (Hitachi Koki Co. Ltd.), and the optical density was measured at 795 nm in a Hitachi 356 spectrophotometer. There was no significant difference between the absorbances of ICG in the presence or absence of CPZ in bile. Bile volume was measured gravimetrically, assuming a density of 1.0 for bile. The procedure for determining ICG in the liver, reported by Paumgartner *et al.* [21] was used as previously described [19]. GOT was determined using a commercial kit (Hepatest A, Daiichi Pure Chemicals Co. Ltd., Tokyo), and LDH was determined by the method of Zimmerman and Weinstein [22]. Bile acid was determined by the 3α-hydroxysteroid dehydrogenase method of Talalay [23] as modified by Javitt and Emerman [24]. Plasma albumin concentration was determined using a commercial kit (Diagnotest A, Daiichi Pure Chemicals Co. Ltd.).

Pharmacokinetic analysis

Parameters in plasma and bile kinetics were calculated with a non-linear iterative least squares method [25], using a Hitachi M-200H digital computer.

Statistical analysis

All means are presented with their standard errors. Student's *t*-test was utilized to determine a significant difference between the control and the CPZ-treated groups, with $P < 0.05$ as the minimal level of significance.

RESULTS

Bolus injection study

Plasma disappearance curves for 120 min after intravenous administration of 3 μmoles (8.5 mg/kg) of ICG are shown in Fig. 1. Typical delays of plasma disappearance were observed in both the CPZ-treated groups. Biliary excretion profiles of ICG are shown in Fig. 2. The biliary excretion rate of ICG was significantly lower in the second collection period (from 10 to 20 min) after injection in the CPZ 10 mg/kg treated rats when compared to the control rats, while in the CPZ 15 mg/kg treated rats significantly lower excretion rates were observed until the third collection period (from 0 to 30 min). The amount of ICG excreted in bile for 4 hr in the control rats was 62.7 ± 0.02% of the dose (N = 6), while those of the CPZ-treated groups were 57.7 ± 0.02% (N = 5) for 10 mg/kg CPZ and 50.3 ± 0.03% (N = 4) for 15 mg/kg CPZ respectively. The time course of plasma disappearance and biliary excretion was found to be described by a four-compartment model (Fig. 3), since the plasma concentration time course was described by a two-compartment model and one more compartment was necessary to connect the plasma and bile data according to the precursor successor rule proposed by Beck and Rescigno [26]. The pharmacokinetic parameters were computed by a non-linear iterative least squares method and are listed in Table 1. Significant decreases were observed in V_2 and CL_{tot} in the CPZ 10 mg/kg treated rats and in k_{34}, V_2 and CL_{tot} in the CPZ 15 mg/kg treated rats, while a significant increase was observed in k_{21} in

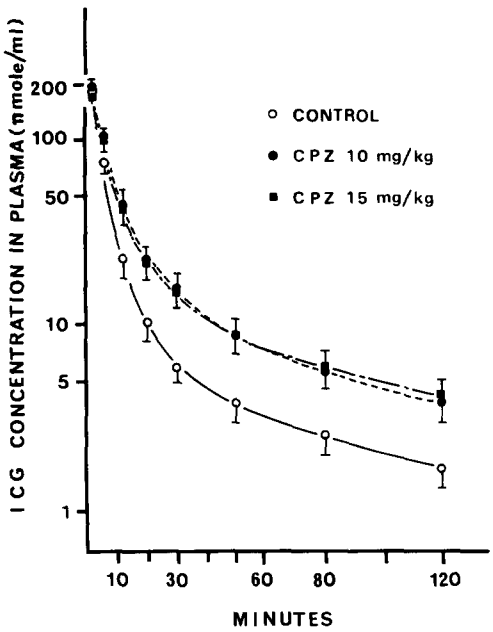

Fig. 1. Plasma disappearance curves of a 3-μmole (8.5 mg/kg) dose of indocyanine green (ICG) after intravenous administration. Each point is the mean ± S.E. of four to six rats. Curves were calculated by an iterative least squares method [25], using a digital computer. Key: (○) control; (●) CPZ, 10 mg/kg; and (■) CPZ, 15 mg/kg.

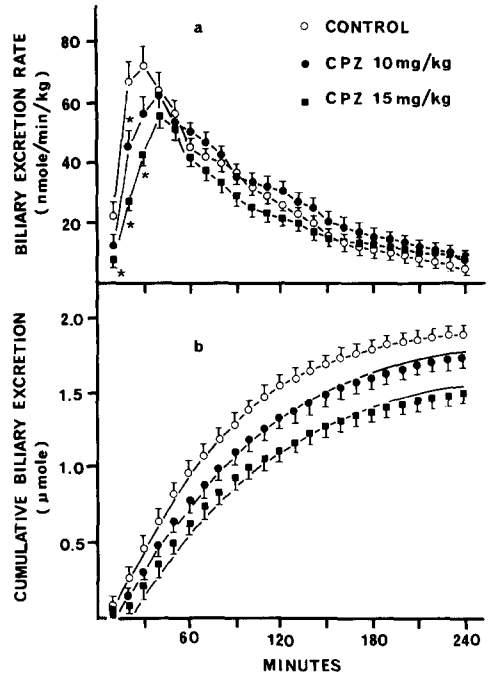

Fig. 2. Biliary excretion profile of ICG after a 3-μmole (8.5 mg/kg) dose administered intravenously. Panel a: biliary excretion rate. Each point is the mean ± S.E of four to six rats. Panel b: cumulative biliary excretion curves. Each point is the mean ± S.E. of four to six rats. Curves were calculated by an iterative least squares method [25], using a digital computer. Key: (○) control; (●) CPZ, 10 mg/kg; (■) CPZ, 15 mg/kg; and (*) significantly different ($P < 0.05$) from the control.

both the CPZ-treated groups; V_1 was not altered. Plasma levels of GOT and LDH at 30 min after intraportal administration of 10 and 15 mg/kg CPZ are shown in Fig. 4, and significant increases were observed in both the CPZ-treated groups.

The liver-to-plasma concentration ratios (K_p) of ICG [20] at 50 min after intravenous administration of a 3-μmole (8.5 mg/kg) dose with or without CPZ administration (15 mg/kg CPZ or physiological saline) are summarized in Table 2; the distribution volumes of the liver (K_pV_L) [20], where V_L is the anatomical liver volume calculated from the liver wet weight assuming a density of 1.0, are also listed in Table 2. Significant ($P < 0.05$) decreases were observed in both K_p and K_pV_L of the CPZ 15 mg/kg treated rats when compared to the control rats.

The effect of CPZ on the bile flow rate is shown in Fig. 5. Bile flow rates decreased immediately after CPZ administration in both the CPZ-treated groups. The minimum bile flow rates were observed during the first collection periods (from 0 to 10 min) after CPZ administration in both the CPZ-treated groups, and the maximum depression ratios of the bile flow rates were 37% of the control in the CPZ 10 mg/kg treated rats and 69% in the CPZ 15 mg/kg treated rats. Subsequently, both bile flow rates returned progressively to the basal levels for 1 hr, and the maximum recovery rates at 1 hr were 94% of that of the control rats in the CPZ 10 mg/kg treated rats but only 72% in the CPZ 15 mg/kg treated rats. The relationship between the bile flow rate and bile acid excretion rate is shown in Fig. 6, according to Ros *et al.* [4]. Since the recovery of the bile flow rate to the basal level in the CPZ-treated groups required approximately 1 hr, as shown in Fig. 5, bile was sampled at 10-min intervals for 50 min in both the CPZ-treated groups. Regression lines calculated by an iterative least squares regression analysis [25] were: $y = 1.034 + 0.0112x$ ($r = 0.896$) in the control rats, which were infused by physiological saline; $y = 0.115 + 0.209x$ ($r = 0.887$) in the CPZ 10 mg/kg

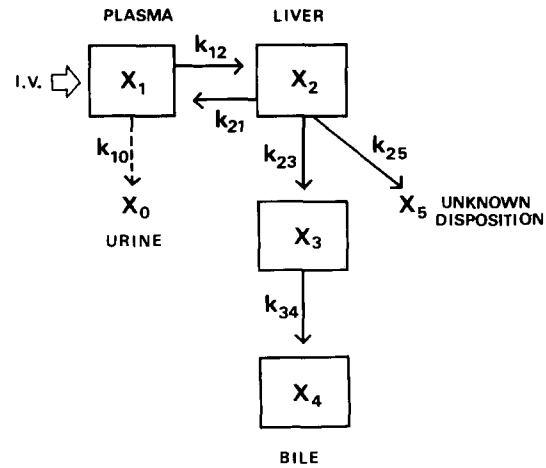

Fig. 3. Schematic illustration of a four-compartment open model obtained from the previous paper [16]. The data of ICG kinetics were fitted in the plasma compartment (X_1) and in the bile compartment (X_4).

Table 1. Pharmacokinetic parameters of ICG calculated with a four-compartment model*

Pharmacokinetic parameters	Control (N = 6)	CPZ (10mg/kg) (N = 5)	CPZ (15 mg/kg) (N = 4)
k_{12} (min^{-1})	0.2430 ± 0.0390	0.1760 ± 0.017	0.1640 ± 0.0210
k_{21} (min^{-1})	0.0058 ± 0.0009	0.0122 ± 0.0023†	0.0120 ± 0.0025†
k_{23} (min^{-1})	0.0105 ± 0.0025	0.0096 ± 0.0019	0.0093 ± 0.0018
k_{25} (min^{-1})	0.0043 ± 0.0009	0.0039 ± 0.0008	0.0038 ± 0.0008
k_{34} (min^{-1})	0.1023 ± 0.0286	0.0582 ± 0.0173	0.0247 ± 0.0065†
V_1 (ml)	10.64 ± 1.08	11.12 ± 0.74	12.01 ± 1.22
V_2 (ml)	428.20 ± 48.40	192.70 ± 38.90†	189.60 ± 24.70†
CL_{tot} ($ml \cdot min^{-1} \cdot kg^{-1}$)§	6.39 ± 0.73	3.95 ± 0.291†	3.78 ± 0.35†

* Results are given as the means ± S.E. Parameters were calculated by a non-linear iterative least squares method [25], using a digital computer (see text). Dose: 3 μmoles (8.5 mg/kg), i.v.

† Significantly different ($P < 0.05$) from the control.

‡ Calculated using the values of R for 8 hr after i.v. administration of ICG listed in Table 3 [19].

§ Total body clearance (CL_{tot}) was calculated using CL_{tot} = dose/AUC, where AUC is the area under the concentration time curve calculated using $AUC = A/\alpha + B/\beta$.

treated rats, and $y = 0.062 + 0.153x$ ($r = 0.963$) in the CPZ 15 mg/kg treated rats. The intercept of ordinate is defined as the bile acid independent fraction of bile secretion [4], and remarkable decreases of the bile acid independent fraction were observed in both the CPZ-treated groups, i.e. 1.034 μmoles/min per g liver in the control rats, 0.115 in the CPZ 10 mg/kg treated rats and 0.062 in the CPZ 15 mg/kg treated rats respectively. The effects of CPZ on the ratio of the total recovery of ICG from the bile to the ICG in the administered dose (R), which was used for the pharmacokinetic analysis [19], and on the total bile volume excreted for 4, 8 and 24 hr after CPZ administration are summarized in Table 3. Only at the 4-hr period were significant decreases ($P < 0.05$) observed in both the ratio and the total bile volume of the CPZ 15 mg/kg treated rats when compared to the control rats; an acute effect of CPZ was not observed in rats at 8 and 24 hr.

Chronic injection study

Some patho-physiological changes resulting from chronic CPZ injection are shown in Table 4. After 3 weeks (treatment of twenty-one doses), body and liver weights were decreased significantly in both chronic CPZ-treated groups (10 and 20 mg/kg per day, i.p. administration). No significant difference was observed in bile flow rate, plasma LDH and GOT, and plasma albumin concentration in either of the chronic CPZ-treated groups. Also, no significant change was shown in the plasma disappearance curve or biliary excretion profile of ICG after 8.5 mg/kg CPZ chronic i.p. administration to either of the CPZ-treated groups (data not shown). The pharmacokinetic parameters were computed by a non-linear iterative least squares method [25] and are listed in Table 5. No significant difference from control was observed in any parameter in either of the chronic CPZ-treated groups.

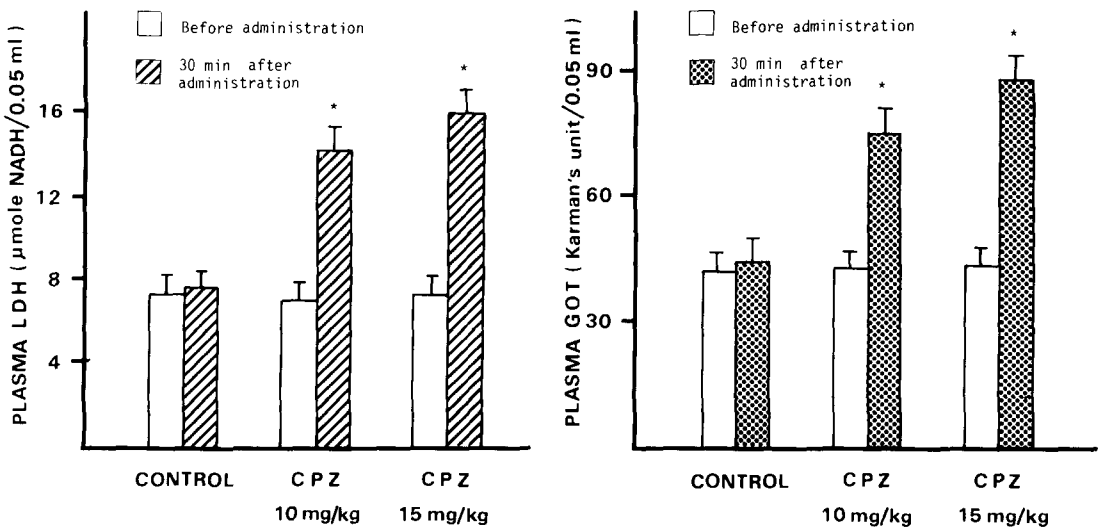

Fig. 4. Plasma LDH (left panel) and GOT (right panel) at 30 min after 10 and 15 mg/kg CPZ, intraportal administration. Each data point is the mean ± S.E. of four to six rats. Key: (*) significantly different ($P < 0.05$) from the control.

Table 2. Liver-to-plasma concentration ratio (K_p) of ICG at the β-phase* and the distribution volume of the liver (K_pV_L)†

	Control (N = 4)	CPZ (15 mg/kg) (N = 4)
K_p	32.2 ± 3.6	18.3 ± 1.8‡
K_pV_L§	403.8 ± 38.6	218.4 ± 23.5‡

* At 50 min after intravenous administration of 3 μmoles (8.5 mg/kg) ICG with or without CPZ administration. $K_p = C_L/C_p$, where C_L is the concentration of ICG in the liver and C_p is that in plasma.

† Results are given as the means ± S.E.

‡ Significantly different ($P < 0.05$) from the control.

§ V_L represents the anatomical liver volume. In this study, we calculated V_L from the liver wet weight assuming a density of 1.0 for liver.

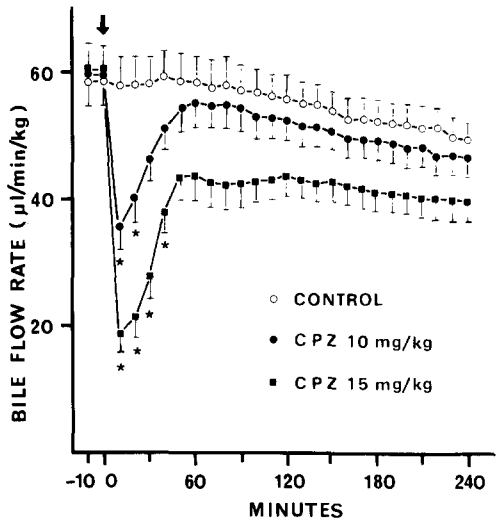

Fig. 5. Effect of CPZ on bile flow rate. CPZ was administered intraportally after a 10-min collection period of bile at the doses of 10 or 15 mg/kg, while the control rats were given physiological saline instead of CPZ (indicated by the arrow). Each point is the mean ± S.E. of four to six rats. Key: (○) control; (●) CPZ, 10 mg/kg; (■) CPZ, 15 mg/kg; and (*) significantly different from the control ($P < 0.05$).

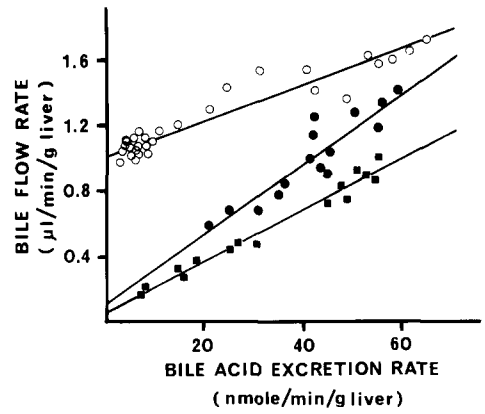

Fig. 6. Relationship between bile flow rate and bile acid excretion rate. Regression lines were calculated by an iterative least squares regression analysis [25]. Key: (○) control rats, infused with physiological saline; (●) CPZ 10 mg/kg treated rats; and (■) CPZ 15 mg/kg treated rats.

DISCUSSION

In this study, we have examined the acute and chronic effects of CPZ on the hepatic transport of ICG in the rat by comparing pharmacokinetic results with biochemical and physiological data in control rats.

The reduction of ICG clearance from the plasma, which is a useful index for diagnostic evaluation of liver function, was observed in rats after bolus intraportal adminstration of 10 and 15 mg/kg CPZ (Fig. 1). Previously we described a pharmacokinetic model for the hepatic transport of BSP [27] and ICG [19] in the rat, in which hepatic blood flow plays a primary role in the initial plasma disappearance of BSP while the permeability of the sinusoidal plasma membrane of the hepatocyte is the rate-determining step in the plasma disappearance of ICG. Furthermore, the product of $k_{12}V_1$ represents the translocation of ICG across the sinusoidal plasma membrane of the hepatocyte. In the present study, no significant change was observed in either k_{12} or V_1 (Table 1), suggesting that bolus intraportal doses of 10 or 15 mg/kg CPZ do not affect the translocation across

Table 3. Ratio (R) of the total recovery of ICG from the bile to the ICG in administered dose and the total bile volume excreted after bolus i.v. administration*

	Sampling periods (hr)	Control (N = 3)	CPZ (10 mg/kg) (N = 3)	CPZ (15 mg/kg) (N = 3)
Ratio (R) of the total recovery of ICG from the bile to ICG in the administered dose	4	0.626 ± 0.013	0.575 ± 0.015	0.517 ± 0.012†
	8	0.683 ± 0.014	0.664 ± 0.022	0.623 ± 0.017
	24	0.718 ± 0.021	0.725 ± 0.019	0.721 ± 0.019
Total bile volume (ml/kg)	4	12.85 ± 0.72	11.87 ± 0.84	9.51 ± 0.71†
	8	24.39 ± 1.48	22.74 ± 1.74	19.24 ± 1.64
	24	65.57 ± 4.95	64.79 ± 5.39	58.37 ± 4.83

* Results are given as the means ± S.E. Dose: 3 μmoles (8.5 mg/kg).

† Significantly different ($P < 0.05$) from the control.

Table 4. Patho-physiological changes after chronic CPZ treatment*

	Control (N = 4)	CPZ (10 mg/kg) (N = 4)	CPZ (20 mg/kg) (N = 4)
Body weight (g)	395.8 ± 9.5	294.2 ± 7.8†	269.4 ± 10.5†
Liver wet weight (g)	19.02 ± 1.53	11.62 ± 0.64†	10.74 ± 0.42†
Bile flow rate ($\mu l \cdot min^{-1} \cdot kg^{-1}$)	73.53 ± 1.15	69.83 ± 2.31	62.62 ± 3.26
Plasma GOT [Karman's unit $\cdot (0.05\ ml)^{-1}$]‡	59.74 ± 4.57	67.56 ± 8.75	75.76 ± 6.53
Plasma LDH [μmoles NADH $\cdot (0.05\ ml)^{-1}$]§	7.78 ± 1.18	7.57 ± 0.83	7.75 ± 0.45
Plasma albumin concn (g/dl)	3.12 ± 0.14	2.81 ± 0.25	2.88 ± 0.23

* Results are given as the means ± S.E.
† Significantly different ($P < 0.01$) from the control.
‡ GOT: glutamic-oxaloacetic transaminase activity.
§ LDH: lactate dehydrogenase activity.

the sinusoidal plasma membrane. Eckhardt and Plaa [13, 14] reported that phenothiazine derivatives cause a marked delay in the plasma disappearance of BSP in mice and rats, and that this effect is due to decreased hepatic blood flow caused by phenothiazine derivatives [15, 16]. The hepatic blood flow, however, may not play a primary role in the plasma disappearance of ICG, as discussed previously [19]. The decrease in k_{34} (Table 1), which is a parameter that is independent of hepatic blood flow, also suggested a toxic effect of CPZ on hepatocytes. Furthermore, the significant decrease in CL_{tot} (Table 1), which was significantly smaller than the hepatic plasma flow calculated from the previous study ($19.9 \pm 4.3\ ml \cdot min^{-1} \cdot kg^{-1}$) [28], suggested a decrease in the parameter for hepatic transport, which is independent of hepatic blood flow. Accordingly, the hemodynamic effect of CPZ on the hepatic transport of ICG may be smaller than on that of BSP, but the ischemia caused by CPZ may affect indirectly the overall hepatic transport of ICG, as suggested by Eckhardt, Plaa and colleagues for BSP [13–16].

On the other hand, a significant increase was observed in k_{21} (Table 1), and this may play a role in the reduction in ICG clearance from the plasma (Fig. 1). Significant increases in plasma LDH and GOT at 30 min after CPZ injection in both the CPZ-treated rats when compared to the control rats (Fig. 4) suggest a change in their efflux from the hepatocyte, as shown previously in isolated rat hepatocytes [10]. The significant increase of k_{21} in both the CPZ-treated rats is due to the significant decrease in V_2, since V_2 was calculated by $V_2 = (k_{12}/k_{21})V_1$ [29], and no significant changes were observed in either k_{12} or V_1. The liver-to-plasma concentration ratio (K_p) of ICG at 50 min (β-phase) after bolus intraportal administration of 15 mg/kg CPZ decreased to approximately one-half that of the control rats (Table 2). The simplest perfusion limited model proposed by Rowland *et al.* [20], which consists of the eliminating organ and the reservoir, can be adapted to the disposition of ICG, since ICG is distributed in the plasma volume without extravascular distribution and is removed exclusively by the liver into the bile without biotransformation [17, 18]. According to this model, the product of $(k_{12}/k_{21})V_1$ ($= V_2$) corresponds to K_pV_E, where V_E is the effective organ volume [usually the anatomical organ volume; i.e. the liver volume (V_L) in this study]. The value of K_pV_L also decreased significantly in the CPZ 15 mg/kg treated rats when compared to that of the control rats (Table 2), and those of the two groups were comparable to the respective values of V_2 listed in Table 1. The values of V_1 in the three groups were similar to the anatomical plasma volume [30]. Con-

Table 5. Pharmacokinetic parameters of ICG calculated with a four-compartment model for chronic CPZ treatment*

Pharmacokinetic parameters	Control (N = 4)	CPZ (10 mg/kg) (N = 4)	CPZ (20 mg/kg) (N = 4)
k_{12} (min^{-1})	0.2625 ± 0.0123	0.2674 ± 0.0112	0.2488 ± 0.0187
k_{21} (min^{-1})	0.0071 ± 0.0005	0.0075 ± 0.0005	0.0077 ± 0.0009
k_{23} (min^{-1})†	0.0097 ± 0.0007	0.0093 ± 0.0008	0.0089 ± 0.0009
k_{25} (min^{-1})†	0.0046 ± 0.0004	0.0044 ± 0.0005	0.0048 ± 0.0006
k_{34} (min^{-1})†	0.0876 ± 0.0074	0.0843 ± 0.0068	0.0798 ± 0.0081
V_1 (ml)	11.94 ± 0.36	12.05 ± 0.33	11.72 ± 0.44
V_2 (ml)	392.6 ± 24.7	384.9 ± 29.5	373.6 ± 32.6
CL_{tot} ($ml \cdot min^{-1} \cdot kg^{-1}$)‡	6.74 ± 0.26	6.44 ± 0.23	6.08 ± 0.37

* Results are given as the mean ± S.E. Parameters were calculated by a non-linear iterative least squares method [25], using a digital computer. Dose: 8.5 mg/kg, i.v.
† Calculated using the values of R of the control for 8 hr after i.v. administration of ICG listed in Table 3 [19].
‡ Total body clearance (CL_{tot}) was calculated using CL_{tot} = dose/AUC, where AUC is the area under the concentration time curve calculated using $AUC = A/\alpha + B/\beta$.

sequently, V_2 may correspond to the liver compartment, and the significant decrease of V_2 in the CPZ-treated groups may be explained by the change of ICG distribution into the liver by CPZ as shown in the remarkable decrease in K_p (Table 2). The significant decrease of the transfer constant from liver to bile, k_{34} in the CPZ 15 mg/kg treated rats, may be explained by the change in the biliary secretion process.

On the other hand, in the chronic injection study no significant change was observed in any of the pharmacokinetic parameters (Table 5) or in the patho-physiological parameters except for the body and liver weights (Table 4). These findings suggest that the CPZ dosage employed in this study (10 and 20 mg/kg i.p. per day for 3 weeks) may not have altered the hepatic transport function that is detectable by ICG disposition and the values of plasma LDH and GOT; this may have been due to the rapid metabolism and excretion of CPZ in the rat ($T_{1/2}$ = 2–3 hr) [31] when compared to that in humans ($T_{1/2}$ = approximately 30 hr) [32]. Robert [33] also reported that CPZ chronic treatment with a dose of 25 mg/kg i.p. per day for 8 days did not produce cholestasis in the rat. Results similar to those shown in this study with ICG were reported previously for BSP disposition in mice after chronic administration of CPZ (3.2 and 6.4 mg/kg per day for 14 days).

A typical cholestasis was produced immediately after the bolus intraportal adminstration of CPZ in both the CPZ-treated groups (Fig. 5), but no change was observed in the bile flow rate in either of the chronic CPZ-treated groups (Table 4). Previously, acute cholestasis by CPZ was reported in isolated perfused rat liver, and a dose-dependent depression of bile secretion as shown in this study (Fig. 5, Table 3) was also observed [5–7]. Ros *et al.* [4] reported a dose-dependent and reversible cholestasis involving suppression of the bile acid dependent and independent flow, inhibition of bile acid synthesis, and impairment of biliary lipid secretion in the rhesus monkey, suggesting bile acid–CPZ interactions and an effect of CPZ on canalicular and other membranes. In this study, (1) a time-dependent recovery of bile flow rate was observed in both the CPZ-treated groups (Fig. 5), (2) at 60 min after intraportal bolus administration of CPZ the bile flow rate returned to the basal level in the CPZ 10 mg/kg treated rats, while in the CPZ 15 mg/kg treated rats the recovery at 60 min was not complete (approximately 70% of the control), and (3) the total bile volume for 4 hr showed a significant decrease when compared to that of the control rats (Table 3). However, at 8 and 24 hr no significant difference was shown in either of the CPZ-treated groups when compared to the control rats (Table 3), and this suggests that the depression of the bile flow rate in the CPZ 15 mg/kg treated rats was also reversible. The decrease of the bile acid independent fraction of bile secretion (Fig. 6) was reported previously in isolated perfused rat liver [5–7] and in the rhesus monkey [4]; this may be attributed to the reversible inhibition of Na^+–K^+ ATPase activity and in part to the toxic effect on membranes as discussed by Ros *et al.* [4]. The inhibition of Na^+–K^+ ATPase by CPZ was also demonstrated by Keeffe *et al.* [12] and Samuels and Carey [34], using isolated rat plasma membrane. On the other hand, the bile acid dependent bile flow decreased in proportion to the mass of bile acid secreted in bile for 50 min after bolus intraportal adminstration of CPZ (Fig. 6), and this reduction of bile acid output may have been due to a dose-related inhibition of both bile acid synthesis and secretion as reported in the rhesus monkey [4].

It has been reported that cholestasis-inducing agents such as BSP [35–37] and ethyl estradiol [38, 39] inhibit both liver mitochondrial oxidative phosphorylation and electron transport, and that 2,4-dinitrophenol (DNP), ethionine and malonate, which also induce cholestasis [40], inhibit the production of ATP in the liver [41]. CPZ has also been reported to inhibit mitochondrial oxidative phosphorylation [11] and the respiratory function of isolated rat hepatocytes [10] and to alter mitochondrial structure [42, 43]. Furthermore, it is suggested that both the bile acid dependent and independent fractions of bile secretion require energy from ATP in the liver [44]. From these findings, the inhibition of mitochondrial respiration and oxidative phosphorylation by CPZ may be one of the most important factors in cholestasis that is induced by intraportal bolus administration as in this study, though the inhibition was time dependent and reversible (Table 3 and Fig. 5). But CPZ chronic treatment (10 and 20 mg/kg i.p. per day for 3 weeks) did not induce cholestasis in the rat (Table 4), and this may have been due primarily to the rapid metabolism and excretion of CPZ in the rat as discussed before. Consequently, cholestasis produced by the acute toxic effect of CPZ may have been responsible for the significant alteration of hepatic transport of ICG shown in Figs. 1 and 2 and Table 1.

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