Influence of Laparotomy on Disposition Kinetics of Diclofenac in CCl₄-Intoxicated Rats

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The effect of the acute hepatic failure induced by CCl₄ on the pharmacokinetics of diclofenac, which is definitely subject to enterohepatic circulation (EHC) in normal rats, was evaluated. This hepatic failure extinguished the secondary peak on the plasma time course which is usually observed in normal rats due to EHC. In the group without EHC by means of bile cannulation, the total clearance (CL) markedly decreased by CCl₄-intoxication from 0.71 l/h/kg down to 0.11 l/h/kg, and mean residence time (MRT) increased from 0.29 h up to 2.8 h. The plasma time curves of the rats with laparotomy and with bile duct-cannulation were almost the same in the CCl₄-intoxicated group. The bile excretion ratio of diclofenac markedly decreased by CCl₄-intoxication from 43% down to 13%. In both groups, 92% of the total diclofenac excreted into the bile was glucuronide. While EHC made area under the curve (AUC) and MRT obviously increase in the CCl₄-free rats, the effect of EHC on these moments was negligible in the CCl₄-intoxicated rats. In the CCl₄-intoxicated condition, the elimination of diclofenac in the rats with laparotomy was considerably slower than that in the rats without laparotomy. The plasma time courses were obviously monoeponential in the former group, while those were almost biexponential in the latter group.

Key words laparotomy; diclofenac; carbon tetrachloride; acute hepatic failure; pharmacokinetics; enterohepatic circulation

It is well known that a liver disease often causes drastic changes in the disposition of drugs. Therefore, for safe drug therapy, it is important to clarify pharmacokinetic changes due to the liver disease. Various effects of liver disease on pharmacokinetics have been investigated. The plasma concentration time curves of drugs which are definitely subject to enterohepatic circulation (EHC) reflect conditions of the liver and intestine. Since the pharmacokinetics of these drugs may also be changed drastically by a hepatic malfunction, it is very important to investigate the effect of the hepatic malfunction on EHC in these drugs. However, there have been few reports concerning the effect of hepatic malfunction on EHC from the aspect of pharmacokinetics. Carbon tetrachloride (CCl₄) is widely used as a common pathological model for a hepatic disease, causing hepatic cellular injury, and the single oral administration of CCl₄ can produce a hepatic centrilobular necrosis. It was demonstrated that there was no significant difference in the plasma concentration curves between the rats with laparotomy and the rats without laparotomy in the CCl₄-free group at a 5% significance level of analysis of variance (ANOVA), so intact rats could be used instead of those with laparotomy for the evaluation of EHC in the control group. However, it has been noticed that many drastic changes, such as a decrease in the clearance of a drug, are sometimes caused by the laparotomy, especially in a disease state. Therefore, we attempted to evaluate the effect of the hepatic malfunction induced by CCl₄ on EHC quantitatively from the change in the time courses of the plasma concentration and the bile excretion, as well as the effect of laparotomy on the disposition kinetics of diclofenac in the CCl₄-intoxicated condition.

MATERIALS AND METHODS

Chemicals Diclofenac sodium and CCl₄ were obtained from Wako Pure Chem. Ind. Ltd. Heparin was obtained from Novo Ind. (Denmark). Commercial test kits (Wako Pure Chem. Ind. Ltd.) were used to determine the plasma transaminase activities (glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT). The other chemicals used for the assay of diclofenac were of guaranteed reagent grade or of HPLC grade.

Animal Experiment Male Wistar rats (170—220 g) were divided into the CCl₄-intoxicated group and the CCl₄-free group. The CCl₄-intoxicated group received a single oral administration of CCl₄ in olive oil (1:1, v/v) at a dose of 4 ml/kg. The rats in the CCl₄-free group received equivalent amounts of olive oil under the same condition. The right jugular vein of each rat was cannulated under light ether anesthesia. The free end of the cannula was filled with heparinized (100 IU/ml) normal saline, which was subcutaneously conducted, and then drawn out at the top of the neck. The animals were then fasted and allowed to recover for 16 h. 24 h after the CCl₄ administration, GOT and GPT activities in the plasma were measured. These values were compared with those in the preceding papers in order to choose which CCl₄-intoxicated rats to use for the practical experiment. The rats which had GOT and GPT activities 10 or more times larger than those of the normal rats were used as the CCl₄-intoxicated rats. The animals in each group were divided into the rats with EHC and those without EHC by bile duct-cannulation with light ether anesthesia. Half of the rats with EHC in each group were treated with laparotomy. After these treatments, each awakening rat was held in the Bollman gauge and received a rapid intravenous administration of diclofenac sodium (5 mg/kg) dissolved in 30% polyethylene glycol solution from the above cannula. Blood samples (0.13 ml) were drawn from the cannula at 5, 15, 30 min, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7 h after the injection. After centrifugation (2000 g, 5 min), the plasma samples were stored at −20 °C until analysis. After each sampling,
the blood volume was replaced with the same volume of saline. In the rats with bile duct-cannulation, bile samples were also collected at 30 min intervals, and immediately frozen at −20 °C.

**Assay Procedure** Diclofenac concentrations in the plasma and in the bile were determined by HPLC methods reported by EL-Sayed et al.12) and by Ribera et al.13) respectively. A high-performance liquid chromatograph (LC-10A, Shimadzu Co., Kyoto, Japan) was used with a stationary phase of Chemosorb 5-ODS-H (150 × 4.6 mm i.d.). The detection wavelength was 280 nm. The flow rate of the mobile phase was 1.0 ml/min. The peak area was recorded on a Chromatopac C-R6A (Shimadzu, Kyoto, Japan). The column temperature was 40 °C. The mobile phase compositions were a pH 3.3 solution composed of H2O : CH3CN (1 : 1, v/v) for analysis of the plasma samples, and a mixture of MeOH–CH3CN–1% CH3COOH (55 : 12 : 33, v/v) for analysis of the bile samples. Calibration lines for both samples were freshly prepared by using plasma and bile spiked with diclofenac at four different concentrations. The correlation coefficients of the calibration lines were greater than 0.999. 300 µl of CH3CN was added to 100 µl of plasma sample to denaturize the plasma protein. After precipitation of the protein by centrifugation (13 min, 2000 g), 50 µl of supernatant was injected into the HPLC system (detection limit: 0.01 µg/ml). To determine the unchanged diclofenac concentration in the bile samples, 400 µl of a solution composed of 1% CH3COOH–MeOH (33 : 67, v/v) was added to 100 µl of the sample to stabilize the conjugate. After vigorous shaking for 10 s and centrifugation for 10 min at 2000 g, 50 µl of the supernatant was injected into HPLC system (detection limit: 0.05 µg/ml). The total (unchanged plus conjugated) diclofenac concentration was determined by using 100 µl of bile sample, to which 100 µl of 0.1 M Na2CO3 was added. The mixture was incubated for 2 h at 40 °C to completely hydrolyze the conjugate. Then, 300 µl of the solution composed of 1% CH3COOH–MeOH (33 : 67, v/v) was added into the above incubated mixture. After being shaken for 10 s and centrifuged for 10 min at 2000 g, 50 µl of the supernatant was injected into the HPLC system.

**Data Analysis** Area under the curve (AUC) and mean residence time (MRT) were calculated by trapezoidal integration, basically with extrapolation to infinite time. However, the moments concerning the rats with EHC in the CCl4-free group were calculated without extrapolation, because of their unstable shape on the declining phase. Since the plasma concentrations in these rats were measured down to about 1% of the maximum concentrations, the truncation error was assumed to be small.

**RESULTS**

Figure 1 shows the mean plasma concentration time courses of diclofenac in the rats of the CCl4-free group (a) and the CCl4-intoxicated group (b). Comparison of the time courses between these groups makes it clear that the acute hepatic failure induced by CCl4 markedly retards the elimination of diclofenac from the plasma in the rats both with and without EHC. All initial plasma concentrations were almost the same. In the CCl4-free group, the secondary peaks on the time courses in the rats with and without laparotomy were obvious around 3 h after administration. In contrast, the time course in the rats with bile duct-cannulation showed almost a monoexponential decay. The time courses in the rats with and without laparotomy nearly coincided with those in the rats with bile duct-cannulation for the first 60 min, and thereafter the former was always greater than the latter in the CCl4-free group. In the CCl4-intoxicated group, the secondary peak on the time course in the rats with laparotomy disappeared, and there was no significant difference in the plasma levels between the rats with laparotomy and with bile duct-cannulation at a 5% significance level of ANOVA. Both time courses declined with almost monoexponential decay. In the CCl4-intoxicated group, the elimination of diclofenac from the plasma in the rats with laparotomy was much slower than that in the rats without laparotomy. The plasma time

![Diagram](image-url)
courses were obviously monophasic in the former group, in contrast to biphasic in the latter.

Figure 2 shows the time courses of the cumulative bile excretion of total drug in the control group (a) and in the CCl₄-intoxicated group (b). Table I presents the ratios of total drug and each species (i.e., unchanged drug or glucuronide) excreted into the bile until the last experimental time in both groups. The bile excretion ratio of total drug markedly decreased by CCl₄-intoxication, from 43.0% down to 12.7%. The ratios of unchanged drug and glucuronide to total drug were about 8% and 92%, respectively, in both groups. No change in the ratio of glucuronide was observed in the CCl₄-intoxicated group.

**Table I. Effects of the Acute Hepatic Failure Induced by CCl₄ on the Biliary Excretion of Diclofenac**

<table>
<thead>
<tr>
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<th>CCl₄-free (n=4)</th>
<th>CCl₄-intoxicated (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab/dose (%)</td>
<td>43.0 (7.67)</td>
<td>12.7 (11.0)</td>
</tr>
<tr>
<td>Ab/Ab (%)</td>
<td>8.15 (1.78)</td>
<td>8.04 (2.23)</td>
</tr>
<tr>
<td>Abc/Ab (%)</td>
<td>91.8 (1.69)</td>
<td>91.8 (2.21)</td>
</tr>
</tbody>
</table>

*Ab, Ab, and Abc represent the amount of total drug, unchanged drug and glucuronide excreted into the bile, respectively. Values are shown by means and (S.D.).*

In Table II, the global moment characteristics are presented for all groups. For the rats without laparotomy in the CCl₄-free group, the moments calculated using the time course data for the first 60 min are also given. It is predicted that the influence of EHC is negligible for the first 60 min. In the CCl₄-free group, MRT increased from 0.287 h up to 0.911 h, and Vₘ increased from 0.190 l/kg up to 0.620 l/kg in the presence of EHC, while the difference in total clearance (CL) was not significant at a 5% significance level of ANOVA. In the CCl₄-intoxicated group, the differences in these moments between the rats with laparotomy and those with bile duct-cannulation were not significant at a 5% significance level of ANOVA. In the rats with bile duct-cannulation, CL decreased by CCl₄-intoxication, from 0.693 l/h/kg down to 0.0949 l/h/kg, and MRT increased from 0.287 h up to 2.76 h. In the rats without laparotomy, CL decreased from 0.854 l/h/kg down to 0.177 l/h/kg, and MRT increased by CCl₄-intoxication, from 0.251 h up to 1.90 h. CL decreased by laparotomy from 0.177 l/h/kg down to 0.106 l/h/kg in the CCl₄-intoxicated group.

**DISCUSSION**

In Fig. 1, the time courses in the rats with and without

**Fig. 2. Cumulative Biliary Excretion of Diclofenac Administered Intravenously (5 mg/kg) to CCl₄-Free Rats (a) (n=4) and CCl₄-Intoxicated Rats (b) (n=3)**

*Each symbol specifies an individual rat.*

**Table II. Global Moment Characteristics of Diclofenac Calculated by Numerical Integration According to Trapezoidal Formula**

<table>
<thead>
<tr>
<th></th>
<th>CCl₄-free rats</th>
<th>CCl₄-intoxicated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With cannulation</td>
<td>With laparotomy</td>
</tr>
<tr>
<td><strong>AUC (µg·h/ml)</strong></td>
<td>7.61 (1.75)</td>
<td>7.91 (0.930)</td>
</tr>
<tr>
<td><strong>CL (l/h/kg)</strong></td>
<td>0.693 (0.159)</td>
<td>0.641 (0.0743)</td>
</tr>
<tr>
<td><strong>MRT (h)</strong></td>
<td>0.287 (0.0579)</td>
<td>1.01 (0.0515)</td>
</tr>
<tr>
<td><strong>Vₘ (l/kg)</strong></td>
<td>0.190 (0.00896)</td>
<td>0.649 (0.0727)</td>
</tr>
</tbody>
</table>

*The moments were calculated using the plasma time course data for the first 60 min. Values are shown by means and (S.D.).*
laparotomy almost coincide with those in the rats with bile duct-cannulation for the first 60 min, and thereafter, the former always becomes greater than the latter in the CCl₄-free group. This difference in the plasma level reflects the reabsorption of diclofenac from the intestine into the systemic circulation. The same result was obtained by Ribera et al. and in our preceding study.⁹,¹³ The influence of EHC which is clear in the CCl₄-free group is negligible in the CCl₄-intoxicated group. It is also known that an acute hepatic failure causes the decrease in albumin concentration, sometimes followed by an increase in the distribution volume of drugs which have a high affinity to albumin.¹⁴ In spite of the extremely high binding of diclofenac to albumin, all the time courses show almost the same initial concentrations. From this result, it was concluded that the change in the distribution volume due to the change in the binding of diclofenac to albumin was not noticed in the present investigation. It was confirmed that intact rats can be used instead of the rats with laparotomy for the evaluation of EHC in the control group in the preceding study,⁹ and the same result was obtained in the present study.

In Fig. 2, the time courses of cumulative bile excretion of total drug in the CCl₄-intoxicated group were fluctuated considerably, compared with those in the CCl₄-free group. Therefore, the experiment was repeated under the same experimental condition, but the fluctuation was also observed. This fluctuation is explained to be due to the variability in the resistance of rats to CCl₄. The biliary concentration of total diclofenac was practically undetectable at 3 h after administration in the CCl₄-free group as reported.⁹,¹³ The biliary excretion was monitored until 7 h in the CCl₄-intoxicated group. The bile excretion ratio of total drug markedly decreased by CCl₄-intoxication from 40% to 10%. Even under the normal condition, only about 20% was reabsorbed from the intestine into the systemic circulation.⁹ Furthermore, in consideration of the gastrointestinal symptoms which accompany the acute hepatic failure induced by CCl₄, it is concluded that EHC is negligible under the CCl₄-intoxicated condition. This corresponds to the fact that the plasma concentration time courses of the rats with laparotomy and with bile duct-cannulation are almost the same in the CCl₄-intoxicated group (Fig. 1). Consequently, when the acute hepatic failure is induced by CCl₄, the decrease in CL and the increase in MRT can be related to the decrease in the biliary excretion. The ratio of glucuronide to the total amount of diclofenac excreted into the bile was not affected by the CCl₄-intoxication. This result coincides with reports⁵,¹⁶ that glucuronidation is relatively preserved in the liver disease state because the hepatic enzyme for glucuronidation (UDP-glucuronosyltransferase) is relatively robust against hepatic intoxication in comparison with the oxidation enzymes. Consequently, the decrease in hepatic clearance is possibly due to the decrease in the liver uptake from the sinusoid or to transport through the hepatocytes rather than the decrease in the glucuronidation. It is also known that the activity of cytochrome P-450 markedly decreases by the hepatic failure induced by CCl₄.¹⁵ In rat, the major portion of diclofenac is excreted into the bile as the glucuronide by ester binding and the glucuronide is generated by the direct glucuronidation of the parent drug.¹⁷ Therefore, it is predicted that the bile excretion ratio in the acute hepatic failure is not affected by the decrease in the activity of cytochrome P-450. This prediction is supported in the present study. MRT markedly increased and Vₘ also increased, but CL was not affected by EHC in the CCl₄-free group. In the CCl₄-intoxicated group, the differences in all parameters (CL, MRT and Vₘ) were not significant in the rats with laparotomy and with bile duct-cannulation at a 5% significance level of ANOVA. This result is consistent with the disappearance of EHC caused by the acute hepatic failure induced by CCl₄ as mentioned. In the rats with bile duct-cannulation, CL markedly decreased and MRT increased by the acute hepatic failure. CL, MRT and Vₘ calculated using the time course data by ejecting the data after 60 min in the rats without laparotomy are assumed to present the disposition parameters without the influence of either EHC or laparotomy under normal conditions. By comparing these values with those in the CCl₄-intoxicated rats without laparotomy, the effect of the acute hepatic failure on the disposition kinetics of diclofenac is estimated. Thus, CL decreased to one fifth the normal level, MRT increased eight times, and Vₘ was almost unaffected by the CCl₄-intoxication. As shown in Fig. 1, the differences in the plasma levels between the rats with and without laparotomy were not significant at a 5% significance level of ANOVA. In contrast to this, the elimination of diclofenac from plasma in the rats with laparotomy was much smaller than that in the rats without laparotomy; the former was monophasic and the latter was biphasic in the CCl₄-intoxicated group. CL decreased by laparotomy in the CCl₄-intoxicated group. Consequently, it is considered that the effect of laparotomy becomes more obvious in the weakened rats with the acute hepatic failure induced by CCl₄. This effect causes the curious contradiction that CL in the rats without laparotomy is much larger than that in those with bile duct-cannulation in the CCl₄-intoxicated condition.

In conclusion, the acute hepatic failure induced by CCl₄ made the retarded elimination of diclofenac from the plasma as well as the markedly decreased bile excretion. Considering the disappearance of the secondary peak on the plasma time course and the level of the bile excretion, it was predicted that the effect of EHC is negligible. In this case, the effect of laparotomy is not negligible. Therefore, the laparotomy is necessary for the precise evaluation of EHC of the drug in the CCl₄-intoxicated condition.

REFERENCES