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# Change of drug excretory pathway by $CCl_4$-induced liver dysfunction in rat

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ABSTRACT

Liver dysfunction affects the pharmacokinetics of drugs. The liver plays an important role in drug excretion as well as drug metabolism and pharmacokinetics. In the present study, the relationship between changes in the cefmetazole (CMZ) excretory pathway and the degree of liver dysfunction induced by $CCl_4$ treatment was investigated. CMZ is mainly excreted as an unchanged form in feces in control rats. Depending on the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), urinary CMZ excretion was increased, whereas fecal CMZ excretion was decreased in rat with liver dysfunction. The AUC of CMZ in rats with severe liver dysfunction was approximately 2-fold higher than that in control rats. Since drug transporters could be involved in drug excretion, changes in the expression of representative hepatic drug transporters in liver dysfunction were investigated by rat DNA microarray. Basolateral solute carrier transporters such as Ntcp, Oct1, and Oatp2 were decreased and basolateral ATP-binding cassette transporters such as Mrp3 and Mrp4 were increased by the $CCl_4$ treatment. On the other hand, canalicular Mrp2 and Bsep were decreased, but Mdr1 was increased. However, the transporter system for CMZ has not been identified yet. In conclusion, we clarified that the fecal and urinary excretory profiles of CMZ were changed clearly depending on the serum AST and ALT levels in liver dysfunction. The changes in the CMZ excretory pathway might be responsible for the changes in the expression of drug transporters.



## 1. Introduction

Liver is the central organ for the detoxification and excretion of many xenobiotics including drugs. Drug excretion may be affected by liver dysfunction, leading to an alteration in the pharmacokinetics [1]. Investigation of the relationship between excretion and liver dysfunction is important for predicting the pharmacokinetics in patients with liver dysfunction to avoid drug adverse reactions. Since impaired liver function would vary due to the severity of liver disease, the pharmacokinetics may become complicated. The purpose of the present study is to elucidate whether the drug excretion

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*Abbreviations:* ABC, ATP-binding cassette; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the serum concentration–time curve; Bcrp, breast cancer resistance protein; Bsep, bile-salt export pump; $CCl_4$, carbon tetrachloride; $CL_{tot}$, total clearance; CMZ, cefmetazole; *K*, elimination rate constant; Mdr, multidrug resistance; Mrp, multidrug resistance associated protein; MRT, mean residence time; Ntcp, $Na^+$-taurocholate co-transporting polypeptide; Oat, organic anion transporter; Oatp, organic anion transporting polypeptide; Oct, organic cation transporter; PCR, polymerase chain reaction; RT, reverse transcription; SLC, solute carrier; VRT, variance of residence time; V, volume of distribution

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profile can be affected depending on the severity of liver dysfunction.

Carbon tetrachloride ($CCl_4$) is a well-known hepatotoxicant and has been frequently used for generating a liver-injured models of rat [2–4]. $CCl_4$ is bioactivated into trichloromethyl free radical by cytochrome P450 2E1 and trichloromethyl may be a cause of liver damage [5]. The liver dysfunction can be assessed by the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations in serum. Therefore, in the present study, the relationship between the AST or ALT levels and urinary or fecal excretion was investigated in rats with $CCl_4$-induced liver dysfunction. We selected a cefamycin antibiotic, cefmetazole (CMZ), as a probe drug because CMZ has been reported to be excreted as an unchanged form in feces in rat [6].

Recently, hepatic transporters have been recognized as a major determinant of drug excretion and disposition [7–9]. There are many uptake or efflux transporters in the hepatic canalicular and sinusoidal membrane. Liver dysfunction has been reported to affect the expressions of some drug transporters [10]. Clarification of changes in the hepatic drug transporters could facilitate understanding the excretory profile in liver dysfunction. In the present study, we also performed DNA microarray analysis to evaluate the changes in the hepatic drug transporters by $CCl_4$-induced liver dysfunction in rat.

## 2. Materials and methods

### 2.1. Chemicals

CMZ and cefazolin were purchased from Sigma–Aldrich (St. Louis, MO). $CCl_4$, Transaminase CII-test Wako, and Creatinine-test Wako were purchased from Wako Pure Chemical Industries (Osaka, Japan). All primers shown in Table 1 were commercially synthesized at Hokkaido System Science (Sapporo, Japan). All other chemicals and solvents were of the highest or analytical grade commercially available.

**Table 1 – Sequence of primers of rat transporter used in the present study**

| Primer | Sequence |
|---|---|
| Mdr1a S[a] | 5′-ATCAACTCGCAAAAGCATCC-3′ |
| Mdr1a AS[a] | 5′-AATTCAACTTCAGGATCCGC-3′ |
| Mdr1b S[a] | 5′-CACTGGTGCCTCTGAGTTGA-3′ |
| Mdr1b AS[a] | 5′-GCACATCTTCATCCACATCCT-3′ |
| Mrp2 S | 5′-CAGTCACGGCTTCCTTTCTG-3′ |
| Mrp2 AS | 5′-AGGTTTCCGCTGGGACTTCT-3′ |
| Mrp4 S[b] | 5′-GACAGTTAGTGTGCCTTGCG-3′ |
| Mrp4 AS[b] | 5′-TGGTGAGAACAGTGCACTGG-3′ |
| Bsep S | 5′-TTACTCCGGAGAATAATGAG-3′ |
| Bsep AS | 5′-AGGGCTGACAGCAAGAATCA-3′ |
| Oat2 S | 5′-AATACTTGCTGAGCTGTGCC -3′ |
| Oat2 AS | 5′-AAACAGCAGCTGTGTCTGGT -3′ |

S, sense primer; AS, antisense primer.
[a] From Theron et al. [12].
[b] From Berezowski et al. [13].

### 2.2. $CCl_4$-induced liver dysfunction in rat

The present study was approved by the Institutional Animal Care and Use Committee of Kanazawa University. Male Sprague–Dawley rats (8 weeks old, SLC Japan, Hamamatsu, Japan) were intraperitoneally injected with $CCl_4$ at a dose of 640 mg/kg (a volume of 2 ml/kg) or corn oil once every 2 days for 45 days. The degree of liver dysfunction was assessed by the serum levels of AST and ALT measured. In addition, renal function was evaluated by the serum creatinine levels.

### 2.3. Excretion and pharmacokinetic of CMZ in $CCl_4$-treated rat

For the excretion study, $CCl_4$- or vehicle-treated rats were intravenously injected with CMZ at a dose of 100 mg/ml/kg at 24 h after the final $CCl_4$ treatment. Urine and feces samples were collected using metabolic cages for 24 h after the administration of CMZ. Before the CMZ treatment, blood samples were collected to measure AST, ALT, and creatinine levels.

For the pharmacokinetics study, $CCl_4$- or vehicle-treated rats were intravenously injected at a dose of 100 mg/ml/kg with CMZ at 24 h after the final $CCl_4$ treatment. Blood samples (0.2 ml) were collected at 0, 15, 30, 45, 60, 90, 120, and 180 min after the CMZ treatment.

### 2.4. Effect of $CCl_4$ discontinuation toward CMZ excretion

In four $CCl_4$-treated rats, the $CCl_4$ treatment was discontinued for 14 days after the 45 day $CCl_4$ treatment. After discontinuation, $CCl_4$-discontinued rats were injected with CMZ at a dose of 100 mg/ml/kg. Urine and feces samples were collected for 24 h.

### 2.5. Quantification of CMZ

The CMZ concentrations in urine, feces, and serum were quantified using high-performance liquid chromatography (HPLC) according to a method described previously with slight modifications [11]. Urine samples (0.1 ml) were diluted with 0.4 ml of distilled water. Feces samples were homogenized with distilled water and centrifuged at 1500 × *g* for 10 min. Serum samples (40 μl) were diluted with 20 μl of distilled water. The diluted urine, the supernatant of the feces, and the diluted serum were treated with 0.5% trichloroacetic acid in methanol containing cefazolin as an internal standard. After incubation at −20 °C for 20 min, the mixture was centrifuged at 15,000 × *g* for 10 min. Then the supernatant was diluted 1:1 with 0.1 M citrate buffer (pH 5.4). Fifty microliters aliquots of the sample were injected into the HPLC with a $C_{30}$-5 μm analytical column (Develosil, 4.6 mm × 150 mm, Nomura Chemical, Aichi, Japan). The mobile phase was methanol:10 mM citrate buffer (pH 5.4) = 13:87 (v/v) for the urine and feces samples or 20:80 (v/v) for the serum samples. The flow rate was 1.2 ml/min. The column temperature was 35 °C. The eluent was monitored at 254 nm.

### 2.6. Pharmacokinetic analysis

The pharmacokinetic parameters for CMZ were estimated from the serum concentration–time data by a moment

analysis. The area under the serum concentration curve from 0.25 to 3 h (AUC) was obtained using the trapezoidal rule. Statistical analyses were performed with GraphPad Instat computer program (GraphPad Software, San Diego, CA) by Student *t*-test.

### 2.7. RNA isolation

Total hepatic RNA was extracted using ISOGEN (Nippon Gene, Tokyo, Japan). Samples were divided into three groups based on the degree of liver dysfunction as follows: control group (AST <50 IU/l; ALT <25 IU/l), mild group (AST 50–600 IU/l; ALT 25–200 IU/l), and severe group (AST >600 IU/l; ALT >200 IU/l). Rats in the control group were treated with corn oil, while rats in the mild and severe groups were treated with $CCl_4$ once every 2 days for 45 days. Equal amounts of total mRNA from three rats in each group were pooled and used for microarray and real-time reverse transcription (RT)-polymerase chain reaction (PCR) analysis.

### 2.8. In vitro amplification and DNA microarray

In vitro amplification and DNA microarray analysis were performed using CodeLink™ Bioarray Perfect System according to the manufacturer's protocol (GE Healthcare Bioscience, Piscataway, NJ). cDNA was synthesized from pooled total RNA (2 μg) by RT reaction. The cDNA was used for in vitro transcription reaction by using biotine-11-UTP (Perkin-Elmer, Wellesley, MA). The labeled cRNA (10 μg) was applied to the DNA microarray (rat whole genome bioarray, GE Healthcare Bioscience). Hybridization was performed at 37 °C for 18 h. After washing, the microarray was stained by streptavidin-Cy 5 (Amersham Bioscience) and scanned in an Agilent G2565BA Microarray Scanner using Agilent Scan Control Software (Agilent Technologies, Palo Alto, CA). The image was analyzed using CodeLink™ Expression Analysis Software (GE Healthcare Bioscience).

### 2.9. Real-time RT-PCR

Hepatic drug transporters (Mdr1a, Mdr1b, Bsep, Mrp2, Mrp4, and Oat2) in rat were quantified by real-time RT-PCR. The sequences of the primers are shown in Table 1. cDNA was synthesized as described previously [14]. PCR was performed using the Smart Cycler (Cepheid, Sunnyvale, CA). The PCR conditions were as follows. After initial denaturing at 95 °C for 30 s, amplification was performed by denaturing at 94 °C for 4 s, and annealing and extension at 64 °C for 20 s for 45 cycles. Amplified products were monitored directly by measuring the increase of the dye intensity of SYBR Green I (Molecular Probes, Eugene, OR) that binds to double-strand DNA amplified by PCR.

## 3. Result

### 3.1. $CCl_4$-induced liver dysfunction in rat

In $CCl_4$-treated rats ($n = 18$), AST ranged from 91 to 3913 IU/l and ALT ranged from 37 to 1200 IU/l at 24 h after the final $CCl_4$ treatment. In control rats ($n = 5$), AST ranged from 26 to 37 IU/l and ALT ranged from 13 to 18 IU/l. The function of kidney was assessed by the creatinine concentrations in serum. There was no statistically significant difference in the serum creatinine concentration between the $CCl_4$-treated ($0.89 \pm 0.22$ mg/dl) and control rats ($0.86 \pm 0.20$ mg/dl). For the following experiments, these rats were divided into three groups as follows: control group ($n = 5$; AST <50 IU/l; ALT <25 IU/l); mild group ($n = 8$; AST 50–600 IU/l; ALT 25–200 IU/l); severe group ($n = 11$; AST >600 IU/l; ALT >200 IU/l).

### 3.2. CMZ excretion in $CCl_4$-treated rat

The relationship between the 24 h-cumulative urinary and fecal excretions of CMZ and the serum levels of AST or ALT are shown in Fig. 1. The excretion was calculated as the percentage of the total CMZ recovery. Fecal excretion was dominant in the control group. In $CCl_4$-treated rat, urinary excretion of CMZ increased, whereas the fecal excretion of CMZ decreased according to the increase of the AST and ALT levels. In $CCl_4$-treated rats, urinary excretion reached a plateau above 600 IU/l of AST or 200 IU/l of the ALT. The slopes of the fitted curves of the urinary and fecal excretions were different between rats with mild and those with severe liver dysfunction. The means of the urinary and fecal excretions, AST, and ALT was summarized in Table 2.

### 3.3. Pharmacokinetics of CMZ in $CCl_4$-treated rat

The serum concentrations of CMZ (Fig. 2) and the pharmacokinetic parameters (Table 3) in $CCl_4$-treated and control rats are shown. The serum concentrations of CMZ in the severe group were higher at all time points than those in the mild and control groups. On the other hand, the serum concentrations of CMZ in the mild group were similar to those in the control group. The AUC value of the severe group was significantly higher than that of the control group ($P < 0.05$). Other pharmacokinetic parameters of the mild and severe groups were not changed statistically, but the $CL_{tot}$ and $V$ values tended to be decreased by liver dysfunction.

### 3.4. Effect of $CCl_4$ discontinuation toward CMZ excretion

Fig. 3 shows the urinary and fecal CMZ excretions in the control, severe (before the $CCl_4$-discontinuation), and the discontinued rats. In the $CCl_4$-discontinued rats, urinary CMZ excretion was decreased while fecal CMZ excretion was increased as compared with severe rats. The excretory pattern of CMZ tended to recover by the $CCl_4$ discontinuation. The AST and ALT levels of $CCl_4$-discontinued rats ($35.3 \pm 3.3$ and $16.8 \pm 2.9$ IU/l, respectively) became similar to those of control rats ($31.1 \pm 4.6$ and $16.1 \pm 2.1$ IU/l, respectively).

### 3.5. Changes of hepatic mRNA expression by liver dysfunction

For the DNA microarray analysis, total RNA was pooled from the livers of three rats in the three groups (control, mild, and severe groups). The mean AST levels were 42.1, 468.7, and 1488.0 IU/l in the control, mild, and severe groups, respectively. Analyses of

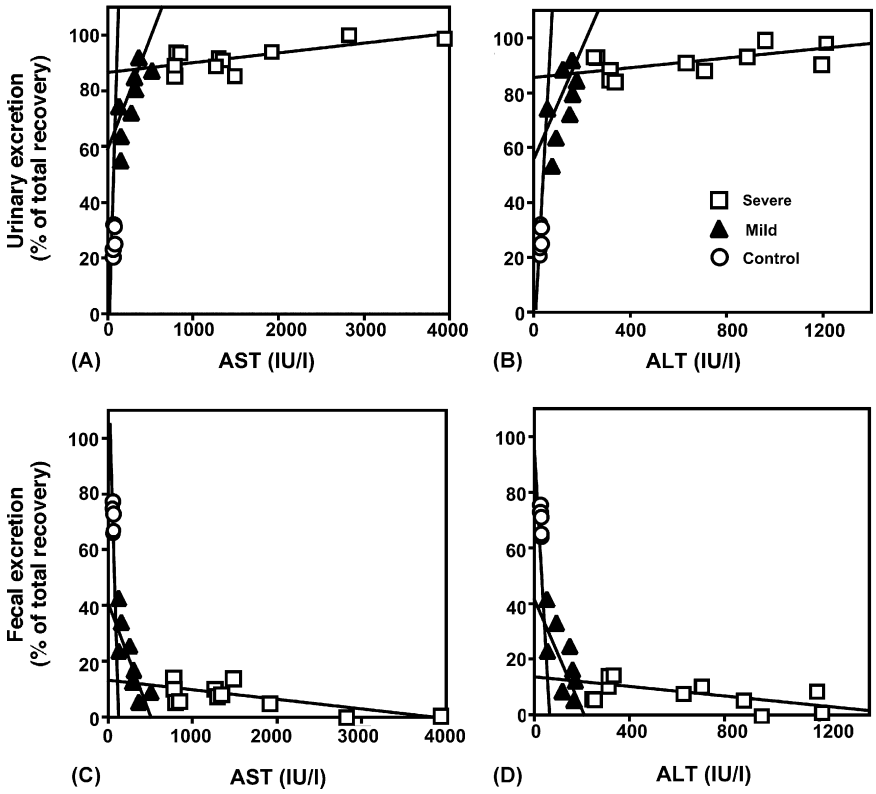


**Fig. 1 – AST and ALT dependent-changes of urinary (A and B) and fecal (C and D) excretion of CMZ in individual rats with $CCl_4$-induced liver dysfunction. Control (○, $n = 5$), AST <50 IU/l, ALT <25 IU/l; mild (▲, $n = 8$), AST 50–600 IU/l, ALT 25–200 IU/l; severe (□, $n = 11$), AST >600 IU/l, ALT >200 IU/l.**

the gene expression profiles identified that 1513 (980 up-regulated and 533 down-regulated) of 11,198 genes were commonly changed above 2-fold in the mild and severe groups. Especially, 8 (5 up-regulated and 3 down-regulated) of 30 genes of the ATP-binding cassette (ABC) transporters and 19 (10 up-regulated and 9 down-regulated) of 141 genes of the solute carrier (SLC) transporters were changed above 2-fold (Table 4). The expression changes of major hepatic drug transporters by the $CCl_4$ treatment are shown in Fig. 4. The expressions of all basolateral SLC transporters were decreased, whereas the expressions of basolateral ABC transporters except Mrp6 were increased depending on the AST levels. In the case of canalicular ABC transporters, the expression of Bsep and Mrp2 were decreased, but those of Mdr1a and Mdr1b were increased by the $CCl_4$ treatment. There was no change in Bcrp expression. To confirm the gene expression profiles of the DNA microarray shown in Fig. 4, real-time RT-PCR was performed in Mdr1a, Mdr1b, Bsep, Mrp2, Mrp4, and Oat2 genes (Fig. 5). The results of the expression profiles of six genes by real-time RT-PCR were almost the same as those by DNA microarray.

## 4. Discussion

In patients with liver disease, reductions in the doses of drugs are sometimes needed in clinical practice [15]. For the optimization of drug treatment, we should pay attention to changes in the pharmacokinetics in liver disease. It is

**Table 2 – Excretion of CMZ and serum levels of AST and ALT in $CCl_4$-treated rat**

| | Control | $CCl_4$-treated | |
|---|---|---|---|
| | | Mild | Severe |
| Urinary excretion (% of total recovery) | 27.6 ± 5.1 | 77.8 ± 12.7** | 92.3 ± 5.1*** |
| Fecal excretion (% of total recovery) | 72.4 ± 5.1 | 22.2 ± 12.7** | 7.7 ± 5.1*** |
| AST (IU/l) | 31.1 ± 4.6 | 220.0 ± 130.3** | 1551.2 ± 987.4*** |
| ALT (IU/l) | 16.1 ± 2.1 | 104.1 ± 48.1** | 633.7 ± 368.8*** |

Data represent the mean ± S.D. (control, $n = 5$; mild, $n = 8$; severe, $n = 11$).
** $P < 0.01$ compared with control rat.
*** $P < 0.001$ compared with control rat.

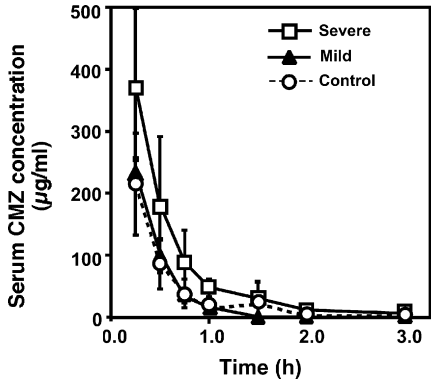


**Fig. 2 – Serum concentration of CMZ in rats with $CCl_4$-induced liver dysfunction. Control (○), AST <50 IU/l, ALT <25 IU/l; mild (▲), AST 50–600 IU/l, ALT 25–200 IU/l; severe (□), AST >600 IU/l, ALT >200 IU/l. Data represent the mean ± S.D. (control, $n = 5$; mild, $n = 3$; severe, $n = 3$).**

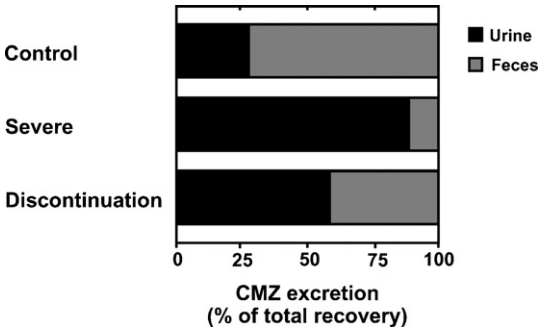


**Fig. 3 – Changes of CMZ excretion after 14-day discontinuation of $CCl_4$ treatment. Control, corn oil treatment once every 2 days for 45 days; severe, $CCl_4$ treatment once every 2 days for 45 days and then showing severe liver dysfunction; discontinuation, non-treatment for 2 weeks after 45-day $CCl_4$ treatment. Data represent the mean ± S.D. (control, $n = 5$; $CCl_4$, $n = 4$; discontinuation, $n = 4$).**

important to predict hepatic function such as biliary excretion. In the present study, we first investigated the relationship between liver dysfunction and the excretory pathway of a drug.

In the present study, liver dysfunction was induced by treatment with a typical hepatotoxicant, $CCl_4$, in rat. Since there were no statistical differences in the serum creatinine concentrations between $CCl_4$-treated and control rats, there appeared to be no renal dysfunction in the present study. In spite of the same experimental condition, liver dysfunction with various AST and ALT levels was generated by $CCl_4$ treatment in the rats. This may be due to differences in the interindividual sensitivity of $CCl_4$, but the reason is still unclear. A large interindividual variability of $CCl_4$-induced cytotoxicity in a histological study of rat livers has been reported [16].

CMZ is predominantly eliminated by the biliary route under normal conditions in rat [6]. In the present study, the urinary excretion was increased and the fecal excretion was decreased by $CCl_4$-induced liver dysfunction. The excretion pathway of CMZ was changed prominently in the range from 50 to 600 IU/l of AST and from 25 to 200 IU/l of ALT, indicating that the excretion of CMZ might be changed in response to an increase in the AST and ALT levels. Urinary excretion of bile acid and drugs is increased by liver disease [17–19], which is assumed to be a type of detoxification. Therefore, a change of the CMZ excretory pathway might also result in detoxification. After 14-day discontinuation of $CCl_4$ treatment, CMZ excretion tended to return to the normal excretory pattern, resembling that in

**Table 3 – Pharmacokinetic parameters of CMZ in $CCl_4$-treated rat**

| | Control | $CCl_4$-treated | |
|---|---|---|---|
| | | Mild | Severe |
| AUC (mg h/l) | 106.6 ± 38.9 | 100.7 ± 10.2 | 210.1 ± 68.5* |
| MRT (h) | 0.6 ± 0.3 | 0.4 ± 0.1 | 0.7 ± 0.2 |
| VRT | 0.9 ± 0.2 | 0.6 ± 0.3 | 0.5 ± 0.2 |
| $CL_{tot}$ (l/h) | 0.5 ± 0.2 | 0.4 ± 0.0 | 0.2 ± 0.1 |
| $K$ ($h^{-1}$) | 1.8 ± 0.7 | 2.5 ± 0.0 | 1.5 ± 0.4 |
| $V$ (l) | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.1 ± 0.1 |

Data represent the mean ± S.D. (control, $n = 5$; mild, $n = 3$; severe, $n = 3$). AUC, area under the serum concentration–time curve; MRT, mean residence time; VRT, variance of residence time; $CL_{tot}$, total clearance; $K$, elimination rate constant; $V$, volume of distribution.
* $P < 0.05$ compared with control rat.

**Table 4 – Expression changes of hepatic transporters in both mild and severe groups**

| Common name | GeneBank ID | Fold induction | |
|---|---|---|---|
| | | Mild | Severe |
| Up regulation | | | |
| Mdr1a | NM_133401 | 4.73 | 8.66 |
| Mdr1b | NM_012623 | 10.18 | 21.50 |
| Mrp1 | NM_022281 | 3.68 | 3.48 |
| Mrp4 | AW141985 | 3.64 | 3.27 |
| Abcg1 | NM_053502 | 2.46 | 3.33 |
| Glast | NM_019225 | 7.56 | 3.24 |
| Cat-1 | NM_013111 | 7.23 | 17.46 |
| Mct7b | BQ200772 | 3.82 | 3.47 |
| Pit-1 | NM_031148 | 2.97 | 3.72 |
| Ant1 | NM_053515 | 5.07 | 4.44 |
| Slc35b2 | NM_199111 | 2.11 | 2.33 |
| Slc39a1 | NM_133315 | 2.01 | 2.06 |
| Down regulation | | | |
| Mrp2 | NM_012833 | 0.40 | 0.35 |
| Mrp6 | NM_031013 | 0.41 | 0.20 |
| Sur | NM_013039 | 0.29 | 0.18 |
| Slc2a5 | NM_031741 | 0.32 | 0.19 |
| Slc6a6 | NM_017206 | 0.41 | 0.33 |
| Gat2 | NM_133623 | 0.40 | 0.22 |
| Nadc1 | NM_031746 | 0.32 | 0.18 |
| Tat1 | NM_138831 | 0.41 | 0.46 |
| Oct | NM_012697 | 0.46 | 0.28 |
| Oat3 | NM_031332 | 0.33 | 0.10 |
| Nckx3 | AY009158 | 0.32 | 0.21 |
| NaPi-Iib | NM_053380 | 0.47 | 0.32 |

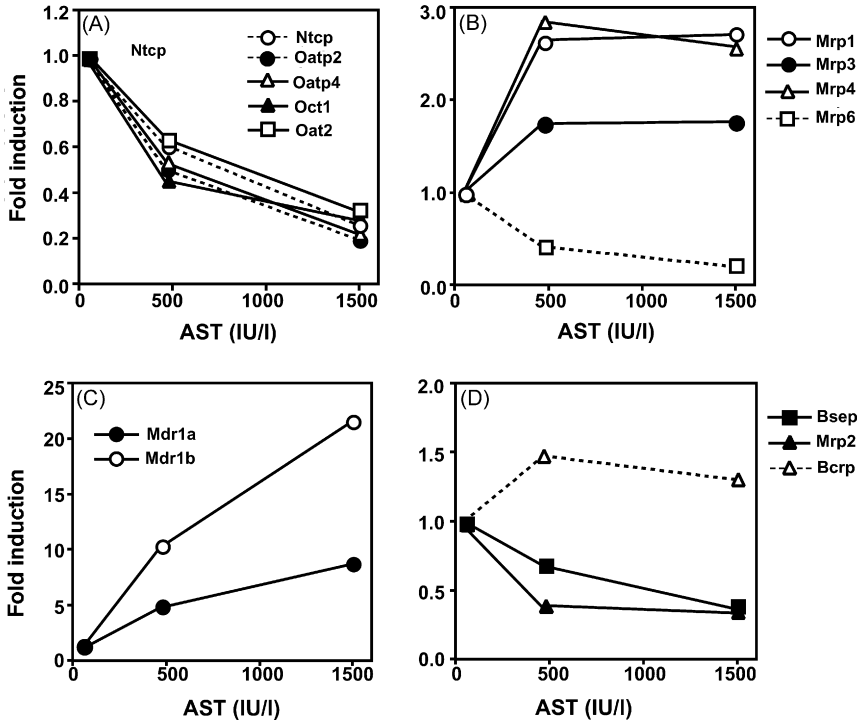


**Fig. 4 – Expression profile of hepatic transporters measured by DNA microarray. (A) Basolateral SLC transporters; (B) basolateral ABC transporters; (C) canalicular Mdr1 transporters; (D) canalicular ABC transporters except Mdr1.**

the control rats. Although the AST and ALT levels were normal in $CCl_4$-discontinued rats, the rate of urinary and fecal excretion was similar but not the same as in the control rats. The AST or ALT levels may be clearly corresponded with changes of the excretion pathway during exacerbation but not during convalescence of the liver dysfunction. Lopez et al. [20] reported that biliary CMZ excretion decreased in cirrhosis rat, but they did not investigate in detail.

Some factors including molecular weight, lipophilicity, and protein-binding rate have been reported to be involved in determining the excretory pathway and rate [21,22]. Recently, hepatic transports have been recognized as an important determinant of the drug disposition. Actually, biliary clearance of a typical P-gp substrate, digoxin, was decreased by administration of a P-gp inhibitor, quinidine, in healthy volunteers [23]. Biliary excretion clearance of the Bcrp substrate, pitavastatin, was decreased in Bcrp knockout mice [24]. It still remains unclear whether CMZ can be transported by an active carrier system. Therefore, DNA microarray was performed to evaluate the changes in the expression of various hepatic transporters by $CCl_4$-induced liver dysfunction in the present study. The expressions of many hepatic transporters were changed by the $CCl_4$ treatment (Table 4). An altered expression of transporters may influence the drug disposition and excretion. In terms of the genes shown in Fig. 4, the changes in expression may depend on the severity of liver dysfunction. The administration of $CCl_4$ resulted in reduced expression of basolateral SLC transporter genes and induced the expression of basolateral ABC transporter genes (Fig. 4). Ntcp (Slc10a1) and Oatp2 (Slc21a5) are uptake transporters in the liver and were reported to be decreased in acute liver dysfunction induced by $CCl_4$ in rat [25]. In the case of canalicular ABC transporters, the expression of Mdr1a and Mdr1b genes was up-regulated, whereas that of Bsep and Mrp2 genes was down-regulated by the $CCl_4$ treatment in the present study. It was reporter that Mdr1 was increased and

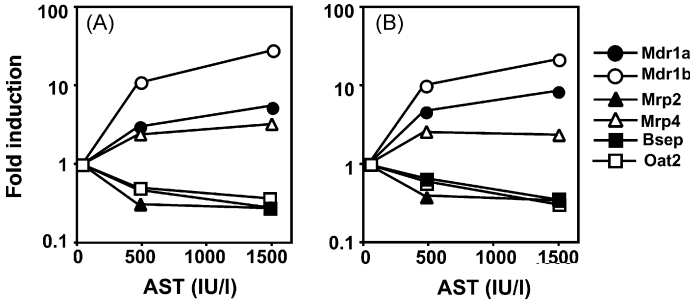


**Fig. 5 – Comparison of expression profile of hepatic transporters between real-time RT-PCR (A) and DNA microarray (B).**

Mrp2 was decreased by a single $CCl_4$ treatment in rat [26,27], which corresponded with our results. Moreover, it was reported that the expression of hepatic Mrp1 and Mrp4 was up-regulated, whereas that of hepatic Ntcp was down-regulated in mice by a single $CCl_4$ treatment [28,29]. Taking the results of the present study and those of previous reports into consideration, alterations in the gene expression of drug transporters may lead to decreased uptake into hepatocytes and increased efflux from hepatocytes. Aleksunes et al. [28,29] suggested that the induction of efflux transporters might be coordinated with the reduction of uptake transporters and this phenomenon would be a defense mechanism for rapid elimination of xenobiotics. Cherrington et al. [30] also indicated that alterations in transporter expression might be related to hepatoprotection, representing a regulation for compensatory process. Our present results would support their hypotheses.

It is still unknown whether CMZ can be transported via drug transporters in the liver, although some cephalosporin antibiotics could be transported by Oat family transporters (Slc22a) [31,32]. In the preliminary study, it was clarified that CMZ was not a substrate for human P-gp, human MRP2, rat Bsep, and rat Bcrp by the measurement of the ATPase activity using the membrane fraction from baculovirus-infected insect cells expressing human P-gp, human MRP2, rat Bsep, or rat Bcrp, respectively (data not shown). Taking the present results into consideration, Mrp4 and/or Mrp3 might be involved in CMZ transport, but further study is needed to clarify the mechanism of CMZ elimination. The increase in urinary excretion and the decrease in biliary excretion of CMZ would be caused by changes in the expression of drug transporters.

In conclusion, liver dysfunction affected the urinary and fecal CMZ excretion in rat in AST- and ALT-dependent manners. This phenomenon could be partly explained by the changes in the expression of drug transporters in liver dysfunction. The present study could provide useful information for the prediction of pharmacokinetics in liver dysfunction.

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