Pharmacokinetics of Cyproheptadine and Its Metabolites in Rats

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To investigate the pharmacokinetics of cyproheptadine (CPH) and its metabolites, the plasma concentration and urinary excretion of CPH and its detectable metabolites were determined after intravenous (i.v.) administration of parent or synthesized metabolites to rats. The plasma CPH concentration–time course was subjected to biexponential calculation following the i.v. administration of CPH, producing the temporal and low plasma concentrations of desmethylcyproheptadine (DMCPH) and the sustained plasma concentrations of desmethylcyproheptadine-epoxide (DMCPhEpo). DMCPH was also eliminated, according to the biexponential equation, after i.v. administration of preformed DMCPH, forming DMCPhEpo in plasma. On the other hand, no detectable DMCPhEpo was found in plasma after the i.v. administration of cyproheptadine epoxide (CPHEpo). All compounds administered had large distribution volumes and were almost entirely excreted as DMCPhEpo in urine; this excretion continued for a long time. However, the urinary excretion pattern of DMCPhEpo after CPHEpo was different from those after CPH and DMCPH. The mean residence times of the epoxidized metabolites estimated from the urinary data were much longer than those from the plasma concentration data, suggesting either a gradual reflux of the metabolites from a tissue depot into systemic circulation under those plasma concentrations close to detection limit, or some interaction which delays excretion into the urine. This study suggests that both metabolic pathways of CPH, through DMCPH and CPHEpo, to DMCPhEpo are possible, but that the demethylation of CPH largely occurs prior to epoxidation; also that the extensive and persistent distribution of DMCPhEpo to tissues may relate to the toxicity of CPH reported in rats.

Keywords cyproheptadine; metabolite; pharmacokinetics; urinary excretion; rat

Cyproheptadine, 1-methyl-4-(5H-dibenzo[a,d]cycloheptenylidene)piperidine (CPH), has antihistaminic and antiserotonin activities, as which is subjected to both N-demethylation and 10,11-epoxidation and N-oxidation in animals. Rats treated with CPH excrete the drug almost entirely as a 10,11-epoxide of desmethylcyproheptadine (DMCPhEpo) and as small amounts of N-demethylated (DMCPH) and epoxidated (CPHEpo) metabolites in urine. These known metabolites of CPH in rats are shown in Fig. 1. On the other hand, the majority of metabolites in mouse and human urine were CPH glucuronide (the quaternary ammonium glucuronide of CPH).

Results from studies of tissue distribution in rats and mice by Wold and Fischer showed that the metabolite(s) of CPH remained in the lung, pancreas, liver and kidney for a long time after the administration of [14C]CPH, although a species difference in CPH disposition was observed between rats and mice. Interestingly, a high dose of CPH causes pancreatic islet cell vacuolization and glucose intolerance only in rats. These drug-related changes were not reported in other laboratory animals. Wold and Fischer have also reported that oral doses of DMCPH to rats produce pancreatic β-cell lesions. These indicate that DMCPH and/or further metabolites may relate to the side effects of CPH. An indicator of CPH-

Fig. 1. Metabolic Pathway of CPH in Rats
Dotted line represents a potential but unproven pathway.

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induced changes in β-cells is the reduction of pancreatic insulin levels that can be observed as early as 24 h after a single administration to rats.1,13) Chow et al. recently reported that DMCPHepo may possibly be involved in the pancreatic β-cell toxicity of CPH, as there was a correlation between the tissue DMCPHepo levels and the toxicity.14)

We have reported the effect of tepipidine on the disposition of CPH and its metabolites after single and repeated administrations in rats, in which the competition of hepatic oxidative metabolism between CPH or its metabolites and tepipidine was observed, resulting in an altered plasma concentration of the metabolites.1,5) Thus, a coadministered drug may produce undesirable side effects from CPH. In spite of the potential significance of CPH metabolites, especially the epoxide metabolites, to pancreatic toxicity, no clear conclusion regarding the metabolic behavior of CPH was fully obtained. Additionally, few studies regarding the contribution of two possible parallel metabolic pathways and the pharmacokinetics of CPH and its metabolites are available. Of the tricyclic drugs forming epoxides, CPH, like carbamazepine, can be transformed into an epoxide across the 10,11-position by human and rats. Evidence exists which indicates that humans may respond to the pancreatic effects of CPH,1,6) but the susceptibility of humans to this action is not clear. Appreciating the toxic potential of epoxides, a thorough knowledge of the pharmacokinetic properties of the metabolites of CPH may help understand their pancreatic toxicity, so that the susceptibility of the human pancreas can be estimated.

In this paper, after intravenous (i.v.) administration of CPH or its synthesized metabolites to rats, a very susceptible species to CPH-induced toxicity, their plasma elimination and urinary excretion were investigated. The aims of this study were to confirm the possible but unproven metabolic routes of CPH to DMCPHepo in the rats, and to discuss the role of the metabolites of CPH, especially DMCPHepo, in CPH-induced toxicity in terms of pharmacokinetic behavior.

Materials and Methods

Materials

CPH hydrochloride was a generous gift of Banyu Pharmaceutical Co. (Tokyo, Japan). Nortriptirine hydrochloride, an internal standard for high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC), was obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). All other chemicals used were of reagent or HPLC grade.

Synthesis and Isolation of Metabolites of CPH

DMCPHepo was synthesized and obtained as hydrochloride salt according to the method of Engelhardt et al.13) CPHepo was synthesized according to the method of Frigerio et al.14) DMCPHepo was obtained from the urine of CPH-treated rats (50 mg/kg, intraperitoneally). Briefly, the pooled urine (0–48 h) was adjusted to pH 10 with 10% NaHCO3 and extracted with dichloromethane. The dichloromethane layer was concentrated in vacuo, and applied to a preparative TLC (silica gel, 0.5 mm, Cica-Merck, Tokyo, Japan). The developing solvent was chloroform-methanol-ethanol (88:7:5). A UV-fluorescent band with Rf 0.6 under UV light was collected and extracted with chloroform-methanol (10:1), and the extract was evaporated.

The products were homogeneous by HPLC and the structures of these metabolites were confirmed using NMR and mass spectrum.

Animal Experiments

Male Wistar rats (270–350 g) had free access to food (MF diet, Oriental Yeast Co., Ltd., Tokyo, Japan) and water during this study. A cannula was implanted in the right jugular vein for drug administration and blood collection under pentobarbitola anesthesia (50 mg/kg, intraperitoneally) one day before the experiment. Each animal was placed in an individual cage until fully recovered from the implanting procedure. Animals received either CPH hydrochloride (4.4 mg/kg as CPH), DMCPH hydrochloride (4.4 mg/kg as DMCPH), or CP Hepo (2.5 mg/kg) intravenously through the cannulation tubing, followed by an injection of 0.2 ml of sterile saline to avoid contamination of the blood samples with traces of the administered dose. Each compound was dissolved in a 40% ethanol-saline solution. Blood samples (0.22 ml) were withdrawn through the cannula into heparinized glass syringes. After each sampling, the blood volume was replaced with the same volume of saline. Immediately, plasma (0.1 ml) was separated by centrifugation at 4 °C and stored at −20 °C until assayed. Urinary excretion studies were also performed under the same treatment as the plasma disposition studies. Animals were individually housed in metabolic cages and urine was collected at appropriate intervals up to 144 h after administration.

Measurement of CPH and Its Metabolites

Determination of CPH and its metabolites in plasma was performed according to the previously reported method.1,5) The limits of detection were ca. 20 ng/ml for all compounds analyzed. For the assay of CPH and its metabolites in urine samples, a GLC method was applied. Briefly, to a urine sample (0.5 ml) were added 100 μl of the internal standard (10 ng/ml of nortriptirine hydrochloride in methanol) and 100 μl of 0.5 N NaOH. The mixture was extracted with 5 ml of dichloromethane by mechanical shaking for 10 min. The organic layer was separated by centrifugation and transferred into another test tube, followed by evaporation. The resultant residue was reconstructed with 50 μl of ethylacetate, and was injected (5–8 μl) into a GLC column. Analyses were performed on a GLC (Hitachi 163 type Gashchromatograph) equipped with a hydrogen flame ionization detector (FID). The column, 1 m x 2 mm (i.d.), was packed with 3% OV-17 on Chromosorb W-AW DMCS (80–100 mesh). Chromatographic conditions were as follows: column oven, detector and injection port temperatures of 250, 285, and 285 °C, respectively; nitrogen flows of 40 ml/min; hydrogen and air inlet pressure of 0.8 and 1.0 kg/cm2, respectively.

Pharmacokinetic Analysis

The pharmacokinetic parameters for the plasma concentration-time curve were calculated using a multi-exponential equation with an iterative non-linear least squares regression program, MULTI.18) The area under the plasma concentration-time curve (AUC), mean residence time (MRT) and distribution volume at steady state (Vss) were calculated according to the common moment analysis.19) The moments were calculated by trapezoidal integration of the time course curve, with extrapolation determined from a monoequponential function using the last three to four data points. The cumulative urinary excretion amount and MRT were also evaluated using urinary excretion data. The computations were carried out on a personal computer (PC-9801XV, NEC Corp., Tokyo, Japan) with programming in BASIC.

Results

Plasma Concentration after Administration of CPH or Its Metabolites

The mean plasma concentration-time curves of the administered drug and detectable metabolites after i.v. administration of CPH or its metabolites are presented in Fig. 2. The time courses of plasma concentration of each of the three intravenously administered compounds were apparently biphasic; thus, the pharmacokinetic evaluation of each compound was performed by a two-exponential model. As shown in Fig. 2A, following i.v. administration of CPH (4.4 mg/kg), only a small fraction of the CPH dose was found in plasma as DMCPH during the initial time stage, and thereafter, no DMCPH was detected; whereas the plasma concentration of DMCPHepo increased progressively to a peak concentration of about 200 ng/ml at 2 h, and the sustained concentration persisted compared to the parent CPH. CPHepo, an epoxidized rather than demethylated metabolite, was not detected in plasma over the time periods after the i.v. administration of CPH.

When receiving 5 mg/kg of CP Hepo intravenously, two of five rats died a few minutes after the dosing. Thus, the dose of CPHepo was reduced to 2.5 mg/kg. DMCPH and CP Hepo were also eliminated from plasma in a biepoxi-
ential manner and were detectable at least until 10 h after the i.v. administration of each preformed metabolite (Figs. 2B and C). All plasma samples collected 24 h after the administration of each of the three compounds included undetectable DMCPHepo concentrations. The pharmacokinetic parameters of CPH and its metabolites are summarized in Table I. The plasma clearance of CPH seemed to be slightly larger than that of DMCPH or CPHepo, but not significant because of an intraindividual variation of CPH clearance. Other parameters were not so different between CPH and its metabolites. The calculated distribution volumes at a steady state, $V_{ss}$, for each of the three compounds were 10 to 231/kg, suggesting an extensive distribution of these compounds to peripheral tissues.

Both CPH and DMCPH produced the consequent metabolite, DMCPHepo, in plasma. The time course of DMCPHepo metabolized from DMCPH (Fig. 2B) was similar to that after CPH administration (Fig. 2A).

### Table I. Pharmacokinetic Parameters of CPH and Its Metabolites after Intravenous Administration of CPH or Preformed Metabolites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPH</th>
<th>DMCPH</th>
<th>CPHepo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(n=6)^a$</td>
<td>$(n=4)^b$</td>
<td>$(n=4)^b$</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>4.4</td>
<td>4.4</td>
<td>2.5</td>
</tr>
<tr>
<td>$A$ (ng/ml)</td>
<td>$1096\pm487$</td>
<td>$631\pm133$</td>
<td>$685\pm159$</td>
</tr>
<tr>
<td>$\alpha$ (h$^{-1}$)</td>
<td>$7.98\pm5.35$</td>
<td>$1.45\pm0.92$</td>
<td>$4.19\pm0.8$</td>
</tr>
<tr>
<td>$B$ (ng/ml)</td>
<td>$161\pm63$</td>
<td>$1.45\pm0.92$</td>
<td>$120\pm90$</td>
</tr>
<tr>
<td>$\beta$ (h$^{-1}$)</td>
<td>$0.38\pm0.28$</td>
<td>$0.21\pm0.14$</td>
<td>$0.20\pm0.0$</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>$2.9\pm1.8$</td>
<td>$4.8\pm3.1$</td>
<td>$3.6\pm0.8$</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng·h/ml)</td>
<td>$720\pm301$</td>
<td>$1207\pm323$</td>
<td>$789\pm357$</td>
</tr>
<tr>
<td>$C_{s0}$ (l/h/kg)</td>
<td>$7.22\pm3.15$</td>
<td>$3.84\pm0.93$</td>
<td>$3.83\pm2.1$</td>
</tr>
<tr>
<td>$V_1$ (l/kg)</td>
<td>$4.35\pm2.47$</td>
<td>$5.80\pm1.60$</td>
<td>$3.29\pm0.8$</td>
</tr>
<tr>
<td>$V_2$ (l/kg)</td>
<td>$18.3\pm6.7$</td>
<td>$10.7\pm2.5$</td>
<td>$18.7\pm8.8$</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>$3.17\pm1.98$</td>
<td>$2.82\pm0.32$</td>
<td>$4.99\pm1.7$</td>
</tr>
</tbody>
</table>

Plasma concentration-time courses were analyzed by a two-exponential equation, $C_t = A e^{-\alpha t} + B e^{-\beta t}$. Values are expressed as the mean±S.D. 

### Table II. Pharmacokinetic Parameters of DMCPHepo after Intravenous CPH or DMCPH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Administered compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPH $(n=6)^a$</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>$8.5\pm2.6$</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng·h/ml)</td>
<td>$2649\pm683$</td>
</tr>
<tr>
<td>$t\cdot MRT$ (h)</td>
<td>$12.4\pm3.8$</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±S.D. 

However, following the administration of CPHepo, DMCPHepo was never detected in plasma (Fig. 2C). Table II shows some pharmacokinetic parameters of DMCPHepo after the administration of CPH or DMCPH.

**Urinary Excretion after Administration of CPH or Its Metabolites** The cumulative urinary excretions of CPH and its metabolites until 144 h after the i.v. administration of each compound are depicted in Fig. 3, where the values for the metabolites are normalized as equivalent to CPH dose of molar base. All compounds administered were excreted almost entirely as DMCPHepo in urine, and the excretion was apparently not completed even at 144 h after administration; that is, a very slight increase in the excretion of DMCPHepo continued. With CPH, an almost negligible amount of CPH (0.4% of CPH dose) and only slight amount of CPHepo (1.1% of CPH dose) were recovered in urine, as shown in Fig. 3A. A negligible amount of DMCPH (0.2% of DMCPH dose) was detected in urine after the i.v. administration of DMCPH (Fig. 3B), whereas a slight amount of CPHepo (2.3% of dose) was excreted as unchanged after the i.v. administration of CPHepo (Fig. 3C). In the case of dosing of CPH and DMCPH, more than 70% of the amount of DMCPHepo excreted was recovered within 48 h (Figs. 3A and B). By contrast, with CPHepo only about 30% of the amount of DMCPHepo excreted was recovered within 48 h, and a secondary increase in excretion was observed 48 to 96 h after dosing (Fig. 3C). Cumulative excreted amounts are shown in Table III, as well as the estimated $MRT$ based on the urinary excretion rate–time curve. The $MRT$ of CPHepo and the total $MRT$
Fig. 3. Time Course Curves of Cumulative Excretion Amounts of CPH and Its Detectable Metabolites after Intravenous Administration of CPH or Its Known Metabolites

(A) ●, CPH following i.v. dose of 4.4 mg/kg of CPH; ▲, CPHepo; ■, DMCPHepo generated from CPH; (B) ●, DMCPH following i.v. dose of 4.4 mg/kg of DMCPH; ■, DMCPHepo generated from DMCPH; (C) ▲, CPHepo following i.v. dose of 2.5 mg/kg of CPHepo; ■, DMCPHepo generated from CPHepo.

| TABLE III. Values of Fraction Excreted in Urine and Mean Residence Time of CPH and Its Metabolites |
|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|
| Administered compound | CPH | DMCPH | CPHepo |
| (n=8) | (n=6) | (n=6) |
| F (%)\[n] | 0.4 ± 0.2 | ND | ND |
| DMCPH | 0.2 ± 0.1 | ND | ND |
| CPHepo | 1.1 ± 0.6 | 2.3 ± 0.9 | 2.3 ± 0.9 |
| DMCPHepo | 45.5 ± 3.5 | 61.4 ± 9.6 | 70.0 ± 22.3 |
| MRT (h) | 6.1 ± 2.7 | ND | 33.7 ± 14.2 |
| t-MRT for DMCPHepo (h)| 21.6 ± 8.4 | 22.1 ± 3.4 | 80.5 ± 19.4 |

Values are expressed as the mean ± S.D. a) Numbers of animals. b) Fraction of dose excreted in urine up to 144 h. ND: not detectable. c) Total MRT from administration of precursor, not intrinsic MRT of DMCPHepo.

Discussion

There are some unclarified points regarding the pharmacokinetics of CPH and its metabolites. Humans are not as vulnerable as rats, as the human excretes a small amount of DMCPHepo in the urine.\[1\] However, an assessment of the pharmacokinetics of the epoxides, such as carbamazepin, must be made to examine their possible relationship to toxicity. To investigate the behavior of the metabolites of CPH and to check the metabolic pathway of CPH proposed in the literature (Fig. 1),\[1\] CPH and its known metabolites, DMCPH and CPHepo, were intravenously dosed to rats. The pharmacokinetic characteristics of DMCPHepo, which is the compound most likely related to pancreas toxicity, were not be completely determined because sufficient amounts of DMCPHepo were not available for administration. The present report, therefore, deals with the measurement of the plasma concentration−and urinary excretion−time courses of DMCPHepo after the injection of CPH, DMCPH and CPHepo, although precise pharmacokinetic analysis is partially limited.

CPH was rapidly and extensively metabolized to DMCPHepo, which is a N-demethylated and oxidized metabolite of CPH. The high plasma concentration of DMCPHepo continued for a long time, compared to the parent CPH, and the transient plasma levels of DMCPH and no detectable CPHepo were observed after the i.v. administration of CPH (Fig. 2A). Similar to CPH dosing, the plasma DMCPHepo level rapidly increased, and thereafter declined gradually following DMCPH dosing (Fig. 2B). On the other hand, no detectable DMCPHepo was seen in plasma over the sampling period after the administration of CPHepo (Fig. 2C). These results suggest that demethylation of CPH occurs rapidly in the liver, and that the demethylation of CPHepo to DMCPHepo proceeds very slowly, based on our results that both DMCPH and CPHepo were excreted predominantly as DMCPHepo in urine (Figs. 3B and C). Frigerio et al.\[2\] have reported that DMCPHepo has been identified, as well as DMCPH and CPHepo, after incubation of CPH with rat liver microsomes. Wald and Fischer\[3\] have reported that after an oral dose of [14C]CPH, unchanged CPH and formed DMCPH decreased rapidly in rat tissues, and that the majority of the radioactivity in the kidney, liver and pancreas, as early as 3 h after the dose, consisted of other metabolites, which closely resemble DMCPHepo. Consequently, CPH is subjected to rapid demethylation initially, followed by oxidation to DMCPHepo.

To further confirm the metabolic pathway of CPH, the urinary excretion of CPH and its metabolites was measured following the same treatment as the plasma disposition study. After the administration of CPH or DMCPH, very small or negligible amounts of unchanged compounds were excreted in urine, and a large amount of DMCPHepo was recovered until 144 h (Figs. 2A and B). This suggests a virtually complete conversion of CPH and DMCPH to DMCPHepo. Interestingly, the excretion of DMCPHepo continued over the sampling periods, contrary to the excretion of the precursors which terminated within 12 h. With CPHepo, CPHepo was also excreted almost entirely as DMCPHepo, along with minor amounts of unchanged
CPHepo (Fig. 3C), despite no detection of DMCPHepo in plasma. A previous study showed that CPH may undergo N-demethylation or epoxidation, and the two resulting metabolites may then be respectively epoxidated or N-demethylated to form the main DMCPHepo. Our results showed that CPHepo was also N-demethylated to DMCPHepo, a possible route which was not unproven. The excretion patterns of DMCPHepo after CPH and DMCPH dosings were qualitatively similar each other. The cumulative excretion pattern of DMCPHepo after i.v. administration of CPHepo, however, was totally different from that after both i.v. CPH and DMCPH. More than 95% of the CPHepo disappeared from the plasma within 10 h after i.v. injection, but less than 10% of the CPHepo dose was recovered from urine at the same intervals. And a clear secondary increase in the cumulative excretion of DMCPHepo was observed after i.v. CPHepo. This indicates that the extensive distribution of CPHepo itself to a peripheral compartment proceeds prior to demethylation, and the demethylation of CPHepo occurs very slowly. Probably, the remaining product is bound to tissues and appears in plasma compartment in undetectably low amounts. Another possibility is the entero-hepatic circulation demonstrated by Hucker et al. 5

In view of the lack of complete information on the pharmacokinetics of DMCPHepo, one can estimate the fraction of CPH demethylated to DMCPH (F_{DMCPHepo-CPH}) from the comparison of the AUC of DMCPHepo following i.v. CPH and DMCPH as follows:

\[
F_{DMCPHepo-CPH} = \frac{AUC_{DMCPHepo-CPH}}{AUC_{DMCPHepo}}
\]

where \( AUC_{DMCPHepo-CPH} \) and \( AUC_{DMCPHepo} \) are the AUCs of DMCPHepo after the administration of CPH and DMCPH, respectively. According to the equation using the data listed in Table II, about 70% of the CPH dose is metabolized to DMCPHepo via DMCPH under the dose tested. This value is close to the value (65%) estimated from the cumulative urinary excretion data according to the equation:

\[
F_{DMCPHepo-CPH} = \frac{A_{e,DMCPHepo-CPH}}{A_{e,DMCPHepo}}
\]

where \( A_{e,DMCPHepo-CPH} \) and \( A_{e,DMCPHepo} \) are the cumulative excretion amounts of DMCPHepo until 144 h sampling after the i.v. administration of CPH and DMCPH, respectively. The fate of the remaining 30—35% of CPH dose is not clear based on the data obtained in this study. Assuming that 30% of the CPH administered would be oxidized to CPHepo, the plasma concentrations of CPHepo above 50 ng/ml of CPHepo should continue during the initial 1 h after the i.v. administration of CPH, with a 70 ng/ml peak level, based on the simulation for a connecting compartment model using the pharmacokinetic parameters of CPH and CPHepo disposition. However, the plasma levels of CPHepo were nonexistent or undetectable after the administration of CPH. Additionally, intravenously administered CPHepo did not produce its demethylated metabolite, DMCPHepo, in plasma. Again, these results strongly suggest that CPH is first demethylated and then oxidized to DMCPHepo. Hucker et al. have reported that CPH was excreted almost equally in urine (31.6%) and feces (29.3%) until 48 h after the i.v. administration of [14C]CPH, and further metabolites such as glucuronide were not found in the urine of rats. Thus, the remaining 30% of CPH dose unrecovered in urine is probably excreted intact and/or as metabolites in feces.

The MRT values of CPHepo and DMCPHepo calculated using the urinary excretion rate-time profile were much longer than those estimated from the plasma data. The MRT obtained from plasma data may have been underestimated, probably due to the fact that these epoxides are taken up in tissue and the flux into the blood is in undetectably low concentrations. This is supported by the result that almost (95%) CPHepo disappeared from plasma within 10 h, while the total amount recovered in urine did not exceed 10% at that time. Another possible reason for this discrepancy in MRT would be due to the delay in the excretion step, although this does not fully explain the mechanism in detail. This speculation is based on the report that the large and extensive accumulation of metabolite(s) other than CPH and DMCPH persists in most tissues, including the kidney. 3 This long accumulation of epoxide metabolite(s) in the tissues, CPHepo and DMCPHepo, may be a cause of toxicity of the CPH-induced pancreatic islets. The results in this study, combined with findings reported by other investigators, suggest that CPH was extensively metabolized via DMCPH formation, followed by oxidation, and that the oxidized metabolites, at least DMCPHepo, were distributed extensively to the tissues, and were retained there for a longer duration than that predicted from the plasma time course of rats. Hintze et al. 13 reported that a 77% decrease in pancreatic proinsulin was observed 6 h after a single 45 mg/kg oral dose of CPH; and at 24 h after a dose of CPH, proinsulin levels were still significantly depressed by 84%. They concluded that an inhibition of proinsulin synthesis is responsible for the depletion of pancreatic insulin. This CPH-induced change in pancreatic ß-cells is considered to be caused by DMCPHepo. Our results, that DMCPHepo is rapidly formed after the administration of CPH and has a larger MRT (21 h) compared to the parent drug, suggest that DMCPHepo is an active metabolite possibly related to the undesirable toxic effect of CPH. A possible reason for the accumulation of DMCPHepo in rats is that the metabolite is not easily conjugated, unlike in mice, in which CPH was excreted as a glucuronic acid conjugate and DMCPHepo did not accumulate. Consequently, the inhibition of conjugation under certain conditions may possibly subject humans to pancreatic toxicity through the same mechanism as in rats. The results in this study also support the possibility of parallel metabolic routes of CPH via DMCPH and CPHepo to DMCPHepo; however, the former may contribute to the CPH metabolism in rats to a larger extent. Thus, CPHepo is a minor factor causing toxicity in rats, although the epoxides are generally considered as toxic products of tricyclic compounds. Rats seem to be a suitable model for the toxicity studies of CPH. Clarification of the mechanism of the delay of excretion of DMCPHepo compared to the plasma profile (i.e., an interaction between the metabolites and tissue components) and the effect of DMCPHepo on pancreatic ß-cell lesions after the direct dosing of DMCPHepo in other species, is a subject for a further
study regarding the toxicity of CPH and its metabolites.

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References