Metabolism of Ipecac Alkaloids Cephaeline and Emetine by Human Hepatic Microsomal Cytochrome P450s, and Their Inhibitory Effects on P450 Enzyme Activities

Takayuki ASANO,* a  HIROTAKA KUSHIDA, a CHIHARU SADAKANE, a KAZUHIISA ISHIHARA, a YOKO WAKUI, a
TOSHIHICO YANAGISAWA, a MASAYUKI KIMURA, a HIDEO KAMEI, a and TAKEMI YOSHIDA b
New Drug Discovery Laboratories R&D Division, Tsumura & Co., Ltd., a 3586 Yoshihara, Aimi-machi, Inashiki, Ibaraki 300–1192, Japan and Department of Biochemical Toxicology School of Pharmaceutical Sciences Showa University b 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–8555, Japan. Received December 13, 2000; accepted March 12, 2001

In this study, we identified the metabolites and the CYP forms that are specifically involved in emetine O-demethylation in human liver microsomes, and cleared the inhibitory potential of cephaeline and emetine on the activity of the major drug-metabolizing CYP enzymes. Incubation of emetine with human liver microsomes yielded three metabolites identified by using HPLC by comparison of the baseline with the authentic sample of cephaeline, 9-O-demethylemetine and 10-O-demethylemetine. CYP3A4 and CYP2D6 were able to metabolize emetine to cephaeline and 9-O-demethylemetine, and CYP3A4 also participated in metabolizing emetine to 10-O-demethylemetine. Cephaeline and emetine inhibited probe substrates metabolism. IC50 for cephaeline against CYP2D6 and CYP3A4 were 121 and 1000 μM, respectively. For the emetine, CYP2D6 and CYP3A4 were 80 and 480 μM, respectively. Inhibition constants (Ki) for both compounds on the CYP2D6 and CYP3A4 activities were determined by graphic analysis of Dixon plots at various concentrations. The obtained Ki values of cephaeline for CYP2D6 and CYP3A4 were 54 and 355 μM, respectively, and the values of emetine were 43 and 232 μM, respectively. We concluded that these in vitro inhibitions of cephaeline and emetine would hardly increase plasma concentrations of co-administered drugs in clinical therapy.

Key words cephaeline; emetine; P450; ipecac syrup; metabolism; human

TJN-119 is an ipecac syrup preparation developed by Tsumura Co., Ltd. as an emergetic medicine for swallowing accidents in Japan. Ipecac syrup as an emetic in the treatment of poisons began in the 1960s in European and North American countries.1) In the U.S.A., ipecac syrup is over-the-counter drug; however, in Japan, it has not been commercially available until now. In a previous paper, we reported on absorption, distribution, metabolism, and excretion of tritium (3H)-labeled cephaeline and emetine, the principal elements contained in ipecac syrup, in rats.2a,b) Those findings indicated that 3H-cephaeline and 3H-emetine remained in tissues for a long term, and the labeled compounds were distributed in the liver at higher concentrations. Therefore, when vomiting has not occurred, various poisons should be of concern in the drug metabolic interaction in the liver with cephaeline and emetine. Moreover, we also found a difference in the metabolic pathway of cephaeline and emetine. Since cephaeline possesses more hydroxygroups than emetine, the compound is likely to be rapidly conjugated and changed into a high polar metabolite by glucuronidation, and is excreted mainly in bile. On the other hand, emetine is demethylated to cephaeline and 9-O-demethylemetine, and is excreted mainly in bile after conjugation with glucuronic acid. In the phase I clinical study, the level of both cephaeline and emetine in plasma increased rapidly after single oral administration and both compounds could be detected in urine up to 8 weeks in more than half of the participants.3) These findings indicate that absorption of these ipecac alkaloids is rapid, but elimination is very slow in humans as well as the rats. However, for cephaeline detected in only human urine for a long term, it is uncertain whether it is excreted as unchanged cephaeline or as a metabolite derived from emetine in administered ipecac syrup.

The objective of this study was to identify the metabolites and the CYP forms that are specifically involved in emetine O-demethylation in human liver microsomes. We also examined the inhibitory potential of cephaeline and emetine, which are the main components of ipecac syrup,4) on the activity of the major drug-metabolizing CYP enzymes in human liver microsomes to clarify the possible drug interactions.

MATERIALS AND METHODS

Chemical and Materials Cephaeline was prepared by Tsumura & Co., Ltd., and emetine was purchased from Fluka Chemical Co., Inc. (Tokyo, Japan). 9-O-Demethylemetine and 10-O-demethylemetine were synthesized by Tsumura & Co., Ltd.5) These chemical structures are shown in Fig. 1. Sulfaphenazole, tranylcypromine, SKF-525A, theophylline, 1-methylxanthine, 1,7-dimethyluric acid, chlor-

Fig. 1. Chemical Structures of Cephaeline, Emetine and Metabolites of Emetine
(a) Cephaeline, (b) R1=H, R2=CH3 emetine, R1=H, R2=CH3; 9-O-demethylemetine, R1=CH3, R2=H; 10-O-demethylemetine.
propamine and propranolol hydrochloride were purchased from Sigma Chemical Co., Inc. (St. Louis, MO, U.S.A.). Furylfplane, S-(+)-mephentoytoine, 4-hydroxytolbutamide, (±)-4’-hydroxymephenitoine, and 1’-hydroxybufuralol maleate were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Ketconazole was purchased from Funakoshi Co., Inc. (Tokyo, Japan). Quinidine, tolbutamide, testosterone and phenobarbital sodium, were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bufuralol hydrochloride was purchased from Gentest Chemical Co., Inc. (Woburn, MA, U.S.A.) and 6β-hydroxytestosterone was obtained from Sumika Chemical Analysis Service, Ltd. (Osaka, Japan). β-Nicotinamide-adenine dinucleotide phosphate (β-NADP⁺), glucose-6-phosphate disodium salt hydrate were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other commercially available reagents and solvents were of analytical or HPLC grade. Human cDNA-expressed CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP4A11 were obtained from Gentest Chemical Co., Inc. Anti-human CYP2D6 and anti-human CYP3A4 were purchased from Daiichi Pure Chemicals Co., Ltd. Frozen human liver microsomes from male and female organ donors were obtained via the International Institute for the Advancement of Medicine, Exton, PA, U.S.A.

In Vitro Metabolism by Human Microsomes The primary incubation medium contained human liver microsomes (0.2 mg/ml) or cDNA-expressed CYP forms (120 nm) in 100 mM potassium phosphate buffer (pH 7.4) containing the NADPH-generating system (5 mM NADP⁺, 50 mM glucose 6-phosphate, 50 mM MgCl₂, and 10 U/ml glucose 6-phosphate dehydrogenase) at 37 °C. The reaction was initiated by the addition of the NADPH-generating system following a 5 min preincubation. The reaction mixture was injected into HPLC following pretreatment.

**Assay with cDNA-Expressed CYP Forms** Microsomes from human baculovirus-infected insect cell lines expressing human CYP1A2 (Lot.6), CYP1B1 (Lot.5), CYP2A6 (Lot.37), CYP2B6 (Lot.45), CYP2C8 (Lot.5), CYP2C9 (Lot.16), CYP2C19 (Lot.8), CYP2D6 (Lot.12), CYP2E1 (Lot.35), CYP3A4 (Lot.54), CYP4A11 (Lot.7) and control microsomes were used. All CYPs except CYP2B6 and CYP4A11 that were used coexpressed (from cDNA) with NADPH-CYP reductase. The final concentrations of microsomes and emetine used were 120 nm and 2 μM, respectively. A 120 min reaction at 37 °C was initiated by adding the NADPH-generating system following a 5 min preincubation. The formation of O-demethylated emetine was indicated for the fluorescence intensity of measuring on HPLC.

**Inhibition of Emetine O-Demethylation** SKF-525A (1—100 μM), which is the potent nonspecific CYP inhibitor, was used to study its inhibitory effect on the O-demethylation activities of emetine (1 μM) in human liver microsomes (0.2 mg/ml). A 15 min reaction at 37 °C was initiated by adding the NADPH-generating system following a 5 min preincubation. For immunoinhibition experiments, different concentrations of anti-CYP2D6 antisera or anti-CYP3A4 antisera and human liver microsomes (0.2 mg/ml) were incubated for 30 min at 37 °C. Emetine (final concentration, 1 μM) was then added, and the mixture was maintained at 37 °C for 5 min. The reaction (15 min) was initiated by the addition of the NADPH-generating system. These results were calculated as a percentage of the control measurements.

**CYP Marker Substrate Assays. Theophylline-O-demethylase Activity** The assay was performed referring to the method described by Relling et al. Theophylline (1.7 nm final concentration) was incubated with human microsomal protein (0.5 mg/ml), 100 mM potassium phosphate buffer, pH 7.4 and NADPH-generating system for 75 min at 37 °C in total incubation volume of 500 μl. The reaction mixture was injected into HPLC following pretreatment.

**Bufuralol 1’-Hydroxylase Activity** The assay was performed referring to the method described by Meier et al. Bufuralol (0.17 mM) was incubated with human liver microsomal protein (0.2 mg/ml), 100 mM potassium phosphate buffer (pH 7.4), and NADPH-generating system for 40 min at 37 °C in total incubation volume of 500 μl. The reaction mixture was injected into HPLC following pretreatment.

**S-(+)-Mephenytoin 4’-Hydroxylase Activity** The assay was performed referring to the method described by Meier et al. S-(+)-Mephenytoin (70 μM) was incubated with human liver microsomal protein (0.4 mg/ml), 100 mM potassium phosphate buffer, pH 7.4 and NADPH-generating system for 60 min at 37 °C in total incubation volume of 250 μl. The reaction mixture was injected into HPLC following pretreatment.

**Testosterone 6β-Hydroxylase Activity** The assay was performed referring to the method described by Arlotta et al. Testosterone (90 μM) was incubated with human liver microsomal protein (0.2 mg/ml), 100 mM potassium phosphate buffer (pH 7.4), and NADPH-generating system for 10 min at 37 °C in total incubation volume of 250 μl. The reaction mixture was injected into HPLC following pretreatment.

**Inhibition Studies** For the inhibition studies by cephæoline and emetine, these compounds were dissolved in methanol to give concentrations of 0.1, 1, 10, 100 mM (only theophylline-O-demethylase activity with cephæoline; 0.0985,
0.985, 9.85, 98.5 mM). Human liver microsomal protein was incubated with the selected marker substrates in the absence and presence of above concentrations of cephaeline or emetine (1—100 μM, only theophylline-O-demethylase activity with cephaeline; 0.0985—98.5 μM, final concentration). Incubation conditions were chosen such that the product formation was linear with respect to both incubation times and protein concentrations, with substrate concentrations being at or below the \( K_m \) for each enzyme. The effects of furafylline, sulphaphenazole, tranylcypromine, quinidine, and ketoconazole, selective inhibitors of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, respectively, were also determined in the same microsomal samples to provide comparisons with inhibitory potentials (IC\(_{50}\)) of cephaeline and emetine towards the individual CYP form.

The \( K_s \) for cephaeline and emetine were determined by using the same pooled microsomal sample. This was achieved by varying the initial substrate concentrations (bufuralol 8, 16 and 32 μM; testosterone 45, 90 and 180 μM) and using several inhibitor concentrations of 10, 50, and 100 μM. The \( K_s \) values were estimated by graphic analysis of Dixon plots. These values were subsequently used as initial estimates for the nonlinear least-squares regression analysis.

RESULTS

Metabolism of Emetine in Human Liver Microsomes

Typical HPLC-chromatogram of the supernatant after incubation of emetine with human liver microsomes, in the presence of the NADPH-generating system, is presented in Fig. 2. Incubation of emetine with human liver microsomes yielded three peaks with the retention time identical to those of cephaeline, 9-O-demethylemetine and 10-O-demethylemetine as compared with the authentic samples. Omission of the NADPH-generating system from the incubation medium completely abolished the demethylation reaction by human liver microsomes, indicating that the process was enzymatic and NADPH-dependent. In addition, SKF-525A (100 μM), a nonspecific potent CYP inhibitor, markedly inhibited (>80%) the NADPH-dependent demethylation of emetine, suggesting that the reaction was mediated by cytochrome P450 (Fig. 3).

Metabolism of Emetine by Various Recombinant CYP Forms

The catalytic properties of each human CYP form for the metabolism of emetine are shown in Fig. 4. CYP2D6 revealed the highest metabolic activity for the generation of 9-O-demethylemetine, whereas this enzyme also showed a significant metabolic activity for the generation of cephaeline. CYP3A4 also exhibited a significant metabolic activity for the generation of these two metabolites and 10-O-demethylemetine. However, other CYP forms exhibited no
Inhibition Analysis of Emetine O-Demethylation

To determine the specific CYP form involved in the biotransformation of emetine, immunoinhibition studies were performed using antihuman CYP antibodies. As shown in Fig. 5, the antihuman CYP3A4 antibody inhibited O-demethylation of emetine to 48.4% (cephaeline), 45.4% (9-O-demethylemetine), and 30.9% (10-O-demethylemetine) at 21 mg IgG/nmol CYP, respectively. The antihuman CYP2D6 antibody inhibited O-demethylation of emetine to 68.1% (cephaeline) and 47.4% (9-O-demethylemetine) at 21 mg IgG/nmol CYP, respectively. However, it did not inhibit O-demethylation to 10-O-demethylemetine.

In Vitro Inhibition Studies on P450 Selective Substrate
(CYP Marker Assay)

The in vitro effects of cephaeline and emetine on the five human CYP activities examined are shown in Table 1. Both compounds produced inhibition of three or four probe substrates metabolism, except theophylline 3-demethylase activity (CYP1A2). IC50 of cephaeline against CYP2C9, CYP2D6 and CYP3A4 was over 1000, 121 and 1000 μM, respectively. In the case of emetine, IC50 for CYP2C9, CYP2C19, CYP2D6 and CYP3A4 was over 1000, 1000, 80 and 480 μM, respectively. Although cephaeline and emetine were able to inhibit the three or four CYP activities, the inhibitory effects of these compounds were very weak when compared to the selective reference inhibitors examined. IC50 values were 4.1, 1.2, 6.3, 0.11, and 0.046 μM against CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 activities by furafylline, sulphaphenazole, tranylcypromine, quinidine, and ketoconazole, respectively.

Further experiments were performed to determine inhibition constants (Ki) for both compounds on the CYP2D6 and CYP3A4 activities. Graphic analysis of Dixon plots at various cephaeline concentrations for each of the two CYP enzyme assays yielded Ki values of 54 and 355 μM for CYP2D6 and CYP3A4, respectively. Similarly, emetine yielded Ki values of 43 and 232 μM for CYP2D6 and CYP3A4, respectively.

DISCUSSION

In our previous metabolism study on ipecac alkaloids in rats, it was confirmed that emetine was demethylated to cephaeline and 9-O-demethylemetine, and excreted in bile by glucuronidation afterwards, while cephaeline was rapidly changed into a high polar metabolite by glucuronidation.25 This study is the first attempt for identifying which CYP forms are responsible for the major metabolic pathway of emetine in humans. In the present study, by using several complementary techniques, we examined the CYP forms involved in the formation of the major metabolite of emetine by human liver microsomes. Cephaeline, 9-O-demethylemetine and 10-O-demethylemetine were formed when emetine
was incubated with human liver microsomes. The findings confirm that emetine is metabolized by demethylation in human liver microsomes as well as rat. These metabolites were generated by the addition of NADPH-generating system, and inhibited by the spike of SKF-525A, indicating that this reaction was catalyzed by cytochrome P450. The emetine O-demethylase activity predominantly occurred by human microsomes which expressed the higher amounts of CYP2D6 and CYP3A4 among the several recombinant CYPs. CYP2D6 produced cephaeline and 9-O-demethylemetine but not 10-O-demethylemetine. Moreover, the generations of cephaeline, 9-O-demethylemetine and 10-O-demethylemetine were observed by CYP3A4 reaction. Inhibition study using the highly specific anti-CYP3A antibody indicated that cephaeline, 9-O-demethylemetine and 10-O-demethylemetine were the products of CYP3A4. The generation of cephaeline, 9-O-demethylemetine and 10-O-demethylemetine was inhibited by highly specific anti-CYP2D6. However, the generation of 10-O-demethylemetine was not influenced by specific anti-CYP2D6. All of these findings suggest that CYP3A4 and CYP2D6 are predominantly involved in the metabolism of emetine to cephaeline, 9-O-demethylemetine, and CYP3A4 also participates for metabolizing emetine to 10-O-demethylemetine.

The present in vitro study also shows that cephaeline and emetine are the inhibitors of CYP2D6 and CYP3A4, in addition to being substrates for these enzymes. When assuming that in vivo plasma substrate concentrations are much lower than the $K_m$ of the metabolizing enzyme, the extent of the inhibition of CYP activities can be predicted as follows\(^\text{(2)}\):

\[
\% \text{ inhibition} = \frac{I}{(1 + K_i)} \times 100,
\]

where $I$ is the concentration of inhibitor at the site of metabolism.

The maximum plasma concentration (cephaeline; 0.01 μM, emetine; 0.02 μM) obtained in a clinical study\(^\text{(2)}\) on this agent has been used, together with the in vitro $K_i$ estimates for each of the major human CYP enzymes, to predict the extent of the inhibition of CYP activities by cephaeline and emetine. These in vitro predictions are based on whole plasma concentrations of cephaeline and emetine in humans since the actual concentrations at the enzyme sites are unknown. In addition, since the plasma concentrations in humans and rats were at very similar levels, the liver concentrations (cephaeline; 0.8 μM, emetine; 3 μM) of the tritium-labeled compounds cephaeline and emetine in rats\(^\text{20}\) are used for evaluating the in vivo inhibitory predictions. The in vitro–in vivo extrapolation indicates that both cephaeline and emetine could have the potential to inhibit the metabolic activities of CYP2D6 and CYP3A4 in the clinical situation, but to a weak extent (ca. 6.6%). The increases of co-administered drug concentrations were only by 1.0–1.1 times (proportional change = 100/100 – % inhibition).

In the case of acute poisoning, the first treatment process is decontamination of poisons from the gut, the second is to promote the excretion of poisons which have already been absorbed, and followed by the antidote administration if available. On the other hand, there have been many cases of infant poisoning with cigarettes every year. Hypnotics psychotropic agents antidepressants and antipyretic analgesics are also well-known drugs that cause acute poisoning. It is known that nicotine and acetaminophen are mainly metabolized by CYP2A6, CYP2B6 and CYP2E1, respectively\(^\text{(13,14)}\). However, they are irrelevant to drug interactions in liver with cephaeline and emetine. Hypnotics, triazolam and brotizolam, are mainly metabolized by the CYP3A family, and the antidepressant selective serotonin reuptake inhibitor is metabolized by CYP2D6.\(^\text{(15–17)}\) Psychotropic agent haloperidol is metabolized by CYP3A4 and CYP2D6.\(^\text{(18)}\) Based on the present results, we presume that these well-known causable drugs of acute poisoning may not influence cephaeline and emetine metabolism by human liver CYPs. Thus, the emetic activities of cephaeline and emetine would be effective even when applied to emergency patients suspected to be poisoned by one of the aforementioned drugs and so on. Additionally, we found that the repeated oral administration of ipecac fluidextract to rats at the dose of 0.07 ml/kg/d (1 ml/kg ipecac syrup equivalent) for 5 d had no effect on microsomal protein content, liver weight and hepatic drug metabolizing enzyme activities (data not shown).

Collectively, we conclude that the lower inhibitory effects of cephaeline and emetine, the principal components of ipecac syrup, on human hepatic microsomal cytochrome P450 activities would hardly cause any drug interactions in clinical use.

REFERENCES