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PHARMACOKINETICS AND DISPOSITION

S. Kudo · M. Odomi

Involvement of human cytochrome P450 3A4 in reduced haloperidol oxidation

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Abstract *Objective*: The present study was conducted to identify in vitro the cytochrome P450(CYP) isoform involved in the metabolic conversion of reduced haloperidol to haloperidol using microsomes derived from human AHH-1 TK +/− cells expressing human cytochrome P450s. The inhibitory and/or stimulatory effects of reduced haloperidol or haloperidol on CYP2D6-catalyzed carteolol 8-hydroxylase activity were also investigated.

Results: The CYP isoform involved in the oxidation of reduced haloperidol to haloperidol was CYP3A4. CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 2E1 were not involved in the oxidation. The k_M value for the CYP3A4 expressed in the cells was 69.7 $\mu mol \cdot l^{-1}$, and the V_{max} was 4.87 $pmol \cdot min^{-1} \cdot pmol^{-1}$ P450. Troleandomycin, a relatively selective probe for CYP3A enzymes, inhibited the CYP3A4-mediated oxidation of reduced haloperidol in a dose-dependent manner. Quinidine and sparteine competitively inhibited the oxidative reaction with a k_i value of 24.9 and 1390 $\mu mol \cdot l^{-1}$, respectively. Carteolol 8-hydroxylase activity, which is a selective reaction probe for CYP2D6 activity, was inhibited by reduced haloperidol with a k_i value of 4.3 $\mu mol \cdot l^{-1}$. Haloperidol stimulated the CYP2D6-mediated carteolol 8-hydroxylase activity with an optimum concentration of 1 $\mu mol \cdot l^{-1}$, whereas higher concentrations of the compound (> 10 $\mu mol \cdot l^{-1}$) inhibited the hydroxylase activity.

Conclusion: It was concluded that CYP3A4, not CYP2D6, is the principal isoform of cytochrome P450 involved in the metabolic conversion of reduced haloperidol to haloperidol. It was further found that reduced haloperidol is a substrate of CYP3A4 and an inhibitor of CYP2D6, and that haloperidol has both stimulatory and inhibitory effects on CYP2D6 activity.

S. Kudo (✉) · M. Odomi
Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan
Fax +81-886-65-6976

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Introduction

Haloperidol is a potent butyrophenone-type antipsychotic drug and is widely used in the treatment of psychoses, especially schizophrenia. Several metabolic pathways of this compound have been identified in animals and humans, indicating the reduction of the molecule at the benzylic carbonyl group [1, 2], oxidative N-dealkylation [3], the formation of pyridinium metabolites [4–7], and conjugations [8].

In humans, reduced haloperidol is one of the main metabolites of haloperidol [9], which is produced by a cytosolic ketone reductase [10, 11]. Because reduced haloperidol is converted back to haloperidol, indicating a metabolic reduction/oxidation cycle for the compound, interconversion between haloperidol and reduced haloperidol have been observed in humans [12–15]. The clinical significance of reduced haloperidol has yet to be clarified [16], although correlations between therapeutic efficacy and the plasma ratio of reduced haloperidol/haloperidol have been discussed [17, 18].

There are several indications based on the results of in vitro and in vivo studies that cytochrome P450 2D6 (CYP2D6) is responsible for the metabolic oxidation of reduced haloperidol to haloperidol [19–22] and that reduced haloperidol is a substrate of CYP2D6 [23]. Moreover, it has been indicated that reduced bromperidol, which is a reduced form of a haloperidol derivative, is oxidized to bromperidol by CYP2D6 [24].

However, in preliminary in vitro studies, we found that reduced haloperidol was not metabolized to haloperidol by human cDNA-expressed CYP2D6. In this study we examined the oxidative reaction of reduced haloperidol using 10 human cDNA-expressed cytochrome P450s, and we identified the CYP isoform responsible for the metabolic oxidation of reduced haloperidol to haloperidol. We further demonstrated the characteristic properties of both reduced haloperidol

and haloperidol on CYP2D6 activity, which was measured using carteolol 8-hydroxylase activity, a selective reaction probe for the enzyme [25].

Materials and methods

Chemicals and reagents

Haloperidol, sparteine sulfate pentahydrate, quinidine sulfate dihydrate, and *p*-aminobenzoic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Reduced haloperidol was obtained from Research Biochemicals International (Natick, Mass., USA). Troleandomycin, β-NADPH, and β-NADH were purchased from Sigma Chemical (St. Louis, Mo., USA). Carteolol hydrochloride, 8-hydroxycarteolol hydrochloride, and biperiden hydrochloride were provided by Otsuka Pharmaceutical (Tokyo, Japan). All other reagents and solvents were of high analytical grade.

Human cDNA-expressed cytochrome P450s

Microsomes derived from human AHH-1 TK + /− cells expressing human cytochrome P450s were purchased from Gentest (Woburn, Mass., USA). The following microsomes expressing cytochrome P450s were used in this study: a control microsome containing a vector (catalogue no.: M101b), CYP1A1 (M111b, 40 pmol P450 $\cdot$ mg^{-1} protein), CYP1A2 (M103c, 93, 128 pmol $\cdot$ mg^{-1}), CYP2A6 (M104r, 193 pmol $\cdot$ mg^{-1}), CYP2B6 (M110a, 77 pmol $\cdot$ mg^{-1}), CYP2C8 (M112r, 10 pmol $\cdot$ mg^{-1}), CYP2C9-cys (M109r, 28 pmol $\cdot$ mg^{-1}), CYP2C19 (M119a, 30 pmol $\cdot$ mg^{-1}), CYP2D6-val (P171, 334, 212 pmol $\cdot$ mg^{-1}), CYP2E1 (M106 k, 210 pmol $\cdot$ mg^{-1}), and CYP3A4 (M107r, 75, 49 pmol $\cdot$ mg^{-1}).

The functional viability of each CYP isoform was assessed by the following reaction probe: CYP1A2-mediated (R)-warfarin 6-hydroxylation with the k_M and V_{max} values of 178 μmol $\cdot$ l^{-1} and 0.81 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450, CYP2C9-cys-mediated tolbutamide methylhydroxylation with the k_M and V_{max} values of 73 μmol $\cdot$ l^{-1} and 2.97 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450, CYP2C19-mediated (S)-mephenytoin 4-hydroxylation with the k_M and V_{max} values of 40 μmol $\cdot$ l^{-1} and 4.99 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450, CYP2D6-val-mediated carteolol 8-hydroxylation with the k_M and V_{max} values of 183 μmol $\cdot$ l^{-1} and 26.1 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450, CYP2E1-mediated chlorzoxazone 6-hydroxylation with the k_M and V_{max} values of 345 μmol $\cdot$ l^{-1} and 11.7 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450, and CYP3A4-mediated testosterone 6β-hydroxylation with the k_M and V_{max} values of 72 μmol $\cdot$ l^{-1} and 38.7 pmol $\cdot$ min^{-1} $\cdot$ pmol^{-1} P450 [25].

Enzyme assays

The formation of haloperidol from reduced haloperidol was assayed as follows. In the experiment to identify the CYP isoform involved in the reaction, a 0.5-ml reaction mixture containing 2 mg $\cdot$ ml^{-1} microsomal protein (1.88 mg $\cdot$ ml^{-1} for CYP2D6), 2 mmol $\cdot$ l^{-1} β-NADPH, 1 mmol $\cdot$ l^{-1} β-NADH, and 100 μmol $\cdot$ l^{-1} reduced haloperidol was incubated for 60 min at 37 °C. In CYP2D6, incubations were also conducted at substrate concentrations of 1 and 10 μmol $\cdot$ l^{-1}. One hundred mmol $\cdot$ l^{-1} Tris-HCl buffer, pH 7.4, was used in the CYP2A6 and 2C9 incubation mixtures, and 100 mmol $\cdot$ l^{-1} phosphate buffer, pH 7.4, was used in all other CYP reaction mixtures, including that for the control microsome. The rate of formation of haloperidol was determined over the reduced haloperidol concentration range of 5–250 μmol $\cdot$ l^{-1} in the reaction mixture containing 1 mg $\cdot$ ml^{-1} (49 pmol P450 $\cdot$ ml^{-1}) of microsomal protein expressing CYP3A4. Incubation mixture (0.45 ml), including cofactors, was preincubated for 2 min, and then reaction was initiated by the addition of 50 μl of microsomal suspension. Incubation was performed in an atmosphere of air for 20 min. The reaction rate was linear with incubation time under these conditions. Kinetic parameters were calculated from the linear regression line obtained using a Lineweaver-Burk reciprocal plot.

Troleandomycin, quinidine, sparteine, biperiden, or carteolol was added to the above reaction mixture at concentrations of 10^{-7}–10^{-3} mol $\cdot$ l^{-1} to investigate the effect of these compounds on the CYP3A4-catalyzed oxidation of reduced haloperidol to haloperidol. To estimate the apparent k_i for quinidine or sparteine on the reaction, the haloperidol formation rate was determined as described above in incubation systems containing 1 mg (49 pmol P450) $\cdot$ ml^{-1} of CYP3A4-expressed microsomal protein and final concentrations of 25–100 μmol $\cdot$ l^{-1} for reduced haloperidol, 0–20 μmol $\cdot$ l^{-1} for quinidine, and 0–1000 μmol $\cdot$ l^{-1} for sparteine. k_i values were calculated from the linear regression line obtained by a Dixon plot.

Assay of carteolol 8-hydroxylation was performed as a selective reaction probe for CYP2D6 activity [25]. Incubation systems contained 0.3 mg (100 pmol P450) $\cdot$ ml^{-1} of CYP2D6-expressed microsomal protein, 2 mmol $\cdot$ l^{-1} β-NADPH, 1 mmol $\cdot$ l^{-1} β-NADH, and 50 mmol $\cdot$ l^{-1} phosphate buffer, pH 7.4, in a final volume of 0.5 ml. Reaction mixtures were incubated at 37 °C for 10 min in an atmosphere of air. The effects of reduced haloperidol or haloperidol were investigated using the above assay of CYP2D6-catalyzed carteolol 8-hydroxylation. In the experiment for haloperidol, the compound was added to the reaction mixture containing 100 μmol $\cdot$ l^{-1} carteolol at a concentration of 10^{-7}–10^{-4} mol $\cdot$ l^{-1} (seven doses), and the reaction was carried out for 10 min. The rate of carteolol 8-hydroxylase activity was determined as described above in incubation systems containing 0.47 mg (99.6 pmol P450) $\cdot$ ml^{-1} of CYP2D6-expressed microsomal protein and final concentrations of 60–360 μmol $\cdot$ l^{-1} for carteolol and 0–10 μmol $\cdot$ l^{-1} for reduced haloperidol. The k_i value was calculated as described above.

All inhibitors and substrates were dissolved in methanol or water. Methanol added to the incubation mixture was 1% (v/v) in a final concentration and no marked decrease of the enzyme activity was observed. Reactions were quenched by adding 0.5 ml of 20% Na_2CO_3 for the assay of reduced haloperidol oxidation or 0.5 ml of 500-μg $\cdot$ ml^{-1} *p*-aminobenzoic acid used as an internal standard in 20% Na_2CO_3 containing 20 mg $\cdot$ ml^{-1} of sodium bisulfite for carteolol 8-hydroxylation.

Terminated samples for reduced haloperidol metabolism were combined with 5 ml of diethyl ether and shaken for 10 min before centrifuging. Haloperidol was completely extracted in this procedure. Samples for carteolol metabolism were combined with 5 ml of ethyl acetate and shaken for 10 min before centrifuging. After centrifuging at 140 × g for 10 min, 4 ml of the organic phase was evaporated to dryness under a stream of nitrogen. Extracted samples were dissolved in 70 or 100 μl of methanol for 8-hydroxycarteolol and haloperidol, respectively, and then analyzed by HPLC.

HPLC analysis

The HPLC apparatus included two Model 6000 A HPLC pumps (Waters), a Model WISP 710B auto sample processor (Waters), a Model 660 solvent programmer (Waters), a Model 486 tunable absorbance detector (Waters) and a Chromatopac C-R3A (Shimadzu). TSK gel ODS-80$_{TS}$ columns (4.6 mm i.d. × 150 mm for haloperidol and 4.6 mm i.d. × 250 mm for 8-hydroxycarteolol, Tosoh) equipped with a TSK guardgel ODS-80$_{TS}$ guard column (3.2 mm i.d. × 15 mm, Tosoh) were used for the analyses. The mobile phase used for haloperidol was a solution of 5% CH_3CN containing 0.03% trifluoroacetic acid as solution A and 80% CH_3CN containing 0.03% trifluoroacetic acid as solution B, and an isocratic elution with 35% solution B in a mixture of solutions A and B was performed. The flow rate was 1.0 ml $\cdot$ min^{-1}, and UV detection was performed at 254 nm. The retention times for haloperidol and reduced haloperidol were about 13 and 9 min, respectively. The calibration curve for haloperidol was established by an absolute standard method with a calibration range of 0.06–

20 $\mu g \cdot ml^{-1}$ ($\gamma = 0.9995$). The mobile phase used for 8-hydroxycarteolol was a solution of 1% acetic acid as solution A and 30% CH_3CN containing 1% acetic acid as solution B, and an isocratic elution with 35% solution B in a mixture of solutions A and B was performed. The flow rate was 0.8 $ml \cdot min^{-1}$, and UV detection was performed at 254 nm. The retention times for 8-hydroxycarteolol and *p*-aminobenzoic acid used as an internal standard were about 10 and 12 min, respectively. The calibration curve for 8-hydroxycarteolol was established by an internal standard method, based on the peak height ratios between 8-hydroxycarteolol and the internal standard with a calibration range of 0.2–20 $\mu g \cdot ml^{-1}$ ($> \gamma = 0.9990$).

Statistical analysis

A 95% confidence interval of the mean value of carteolol 8-hydroxylase activity in the absence and presence of various concentrations of haloperidol was constructed using an SAS system with version 6.12 computer software (SAS Institute, Cary, N.C., USA).

Results

Identification of CYP isoforms

Rates of formation of haloperidol by CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 at substrate concentrations of 100 $\mu mol \cdot l^{-1}$ are shown in Fig. 1. All CYP isoforms except CYP2C9 were observed to have some catalyzing activity on the oxidation of reduced haloperidol. The primary CYP isoform involved in the reaction was CYP3A4, having a catalyzing activity of 4.495 $nmol \cdot h^{-1} \cdot mg^{-1}$ microsomal protein. Activities of the other CYP isoforms, including CYP2D6, were similar to those of the control microsome containing a vector.

In CYP2D6, catalyzing activity in the reaction was not observed at substrate concentrations of both 1 and 10 $\mu mol \cdot l^{-1}$, showing an activity of less than 0.042 $nmol \cdot h^{-1} \cdot mg^{-1}$ protein.

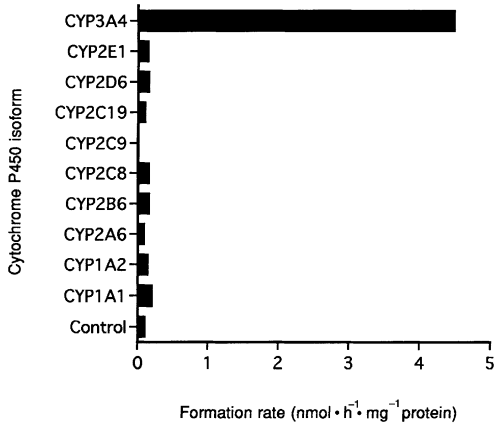

Fig. 1 Catalytic properties of human cDNA-expressed cytochrome P450 on the oxidation of reduced haloperidol to haloperidol

Kinetic parameters for haloperidol formation

Kinetic plots of the formation of haloperidol by the catalyzing activity of CYP3A4 versus the concentration of reduced haloperidol are shown in Fig. 2. Apparent k_M and V_{max} values, determined by Lineweaver-Burk representation, were 69.7 $\mu mol \cdot l^{-1}$ and 4.87 $pmol \cdot min^{-1} \cdot pmol^{-1}$ P450, respectively.

Effects of various chemicals on haloperidol formation

Effects of various chemicals on the CYP3A4-catalyzed oxidation of reduced haloperidol to haloperidol were examined at a concentration range of 10^{-7}–10^{-3} $mol \cdot l^{-1}$. The results are shown in Table 1.

Troleandomycin was a potent inhibitor of the oxidative reaction, dose-dependently inhibiting the reaction at concentrations above 1×10^{-7} $mol \cdot l^{-1}$. Quinidine and biperiden at a concentration of 1×10^{-4} $mol \cdot l^{-1}$ inhibited the formation of haloperidol by 60.6% and 62.7%, respectively, compared to the control value. Sparteine also inhibited the reaction by 20.4% at the high concentration of 1×10^{-3} $mol \cdot l^{-1}$. No inhibition of the CYP3A4-catalyzed oxidation of reduced haloperidol by carteolol was observed.

For quinidine and sparteine, the inhibitory kinetics were investigated using CYP3A4-expressed microsomes to determine the apparent k_i value. Dixon plot representation was used to calculate the apparent k_i value, which was 24.9 $\mu mol \cdot l^{-1}$ for quinidine and 1390 $\mu mol \cdot l^{-1}$ for

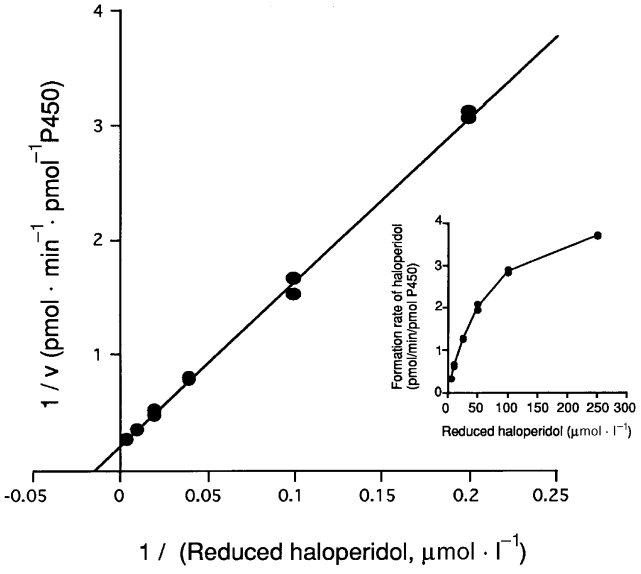

Fig. 2 Lineweaver-Burk plot of the initial velocity of haloperidol formation by CYP3A 4-expressing microsomes. Data points were calculated from the results of the inserted graph at the concentration range of 5–250 $\mu mol \cdot l^{-1}$ of reduced haloperidol. The linear regression line was $y = 14.3332x + 0.2055$ ($\gamma = 0.9993$), yielding an estimate for k_M of 69.7 $\mu mol \cdot l^{-1}$, and for V_{max} of 4.87 $pmol \cdot min^{-1} \cdot pmol^{-1}$ P450

Table 1 Effects of various chemicals on CYP3A4-catalyzed oxidation of reduced haloperidol to haloperidol. A quantity of 100 μmol·l^{-1} of haloperidol was incubated with 1 mg·ml^{-1} of microsomal protein in the assay medium. The control activities were 2.30 and 2.85 nmol·min^{-1}·pmol^{-1} P450 for carteolol, biperiden, and troleandomycin, and quinidine and sparteine inhibitory assay, respectively. Enzyme incubations and metabolite analysis were carried out in duplicate, and the data are expressed as the mean. The values in parentheses are expressed as % of stimulation

Compound	% of Inhibition or (stimulation)				
	Concentration (mol·l^{-1})				
	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
Carteolol	(6.3)	(7.9)	(6.7)	(4.2)	[a]
Biperiden	(5.6)	(0.7)	20.4	62.7	–
Troleandomycin	8.3	50.5	89.4	93.8	–
Quinidine	(4.6)	(8.6)	15.3	60.6	–
Sparteine	–	(5.2)	(5.8)	(7.2)	20.4

[a] Not done

sparteine, indicating a competitive inhibition by each compound of the haloperidol formation by CYP3A4.

Effects of reduced haloperidol and haloperidol on carteolol 8-hydroxylation

The effects of reduced haloperidol and haloperidol on CYP2D6 activity were investigated using carteolol 8-hydroxylation, a selective reaction probe for the enzyme activity.

The Dixon plot illustrating the inhibition of carteolol 8-hydroxylation by reduced haloperidol is shown in Fig. 3. Reduced haloperidol competitively inhibited CYP2D6-catalyzed carteolol 8-hydroxylase activity with a k_i value of 4.3 μmol·l^{-1}. The effects of haloperidol on the enzyme activity is shown in Fig. 4. The mean value

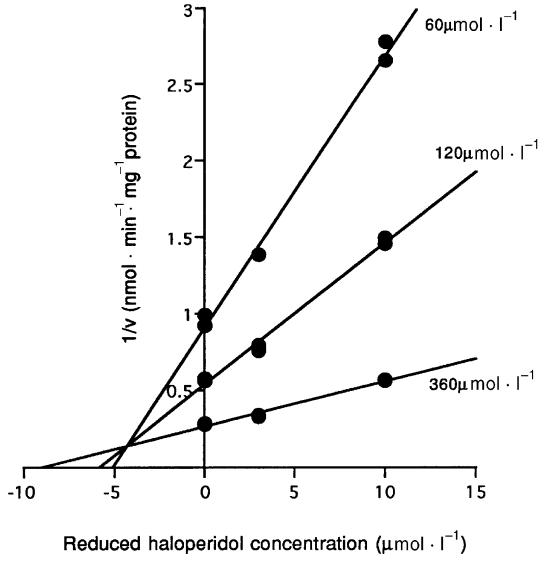

Fig. 3 Dixon plot for the inhibition, by reduced haloperidol, of carteolol 8-hydroxylase activity catalyzed by CYP2D6-expressing microsomes

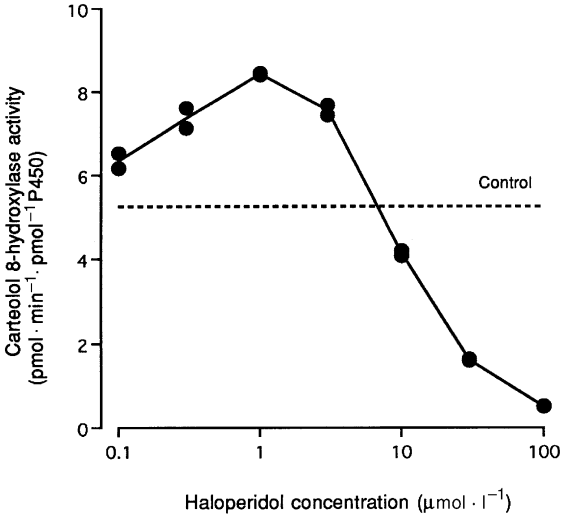

Fig. 4 Stimulatory and inhibitory effects of haloperidol on carteolol 8-hydroxylase activity in CYP2D6-expressing microsomes

of the activity in the absence and presence of various concentrations of haloperidol was 5.15 pmol·min^{-1}·pmol^{-1} P450 with a 95% confidence interval of 2.74–7.55 pmol·min^{-1}·pmol^{-1} P450. The enzyme activity of the control (absence of haloperidol) was 5.25 pmol·min^{-1}·pmol^{-1} P450, showing a similar value to the calculated mean. Carteolol 8-hydroxylase activity with 1 μmol·l^{-1} haloperidol was 8.42 pmol·min^{-1}·pmol^{-1} P450, showing statistically high. The enzyme activity with 30 μmol·l^{-1} haloperidol was 1.61 pmol·min^{-1}·pmol^{-1} P450, showing statistically low. These results indicate that haloperidol has both stimulatory as well as inhibitory effects on CYP2D6 activity.

Discussion

To identify the CYP isoform(s) involved in the metabolic conversion of reduced haloperidol to haloperidol, 10 isoforms of cDNA-expressed human cytochrome

P450 were examined. Only CYP3A4 formed amounts of haloperidol in excess of the background rates obtained with a control microsome. This result indicates that only one isoform of cytochrome P450 is involved in the oxidation of reduced haloperidol to haloperidol. The lack of measurable metabolism with CYP2D6 was conclusive, because the catalytic activity of this CYP isoform was low, being similar to that of the control at a relatively high substrate concentration of 100 $\mu mol \cdot l^{-1}$ and was not observed at substrate concentrations of both 1 and 10 $\mu mol \cdot l^{-1}$.

It has been shown by the manufacturer of cDNA-expressed human cytochrome P450s that human AHH-1 TK +/− cell line contains native CYP1A1. The oxidative activity of reduced haloperidol by CYP1A1-expressing microsomes was comparable to that of the control microsomes. This indicates that the oxidative activity observed in the control microsomes was not due to native CYP1A1 in the cell line. Thus, it was considered that an enzyme excluding cytochrome P450 in the microsomes of the cell line might involve the oxidation of reduced haloperidol to haloperidol at high substrate concentration.

The k_M, V_{max}, and intrinsic clearance for the rate of formation of haloperidol from reduced haloperidol by CYP3A4 were 69.7 $\mu mol \cdot l^{-1}$, 4.87 $pmol \cdot min^{-1} \cdot pmol^{-1}$ P450, and 70 $ml \cdot min^{-1} \cdot pmol^{-1}$ P450, respectively.

Identification of the CYP isoform involved in the metabolic conversion of reduced haloperidol to haloperidol was first discussed by Tyndale et al. [19]. They examined the oxidative reaction in vitro using microsomal preparations of human liver and concluded that the sparteine/debrisoquine-metabolizing isoenzyme P450 2D6 contributed to the oxidation. This conclusion was based on the observation that the oxidative activities of reduced haloperidol by liver microsomes were inhibited by both a low concentration of quinidine with a k_i value of 0.5 $\mu mol \cdot l^{-1}$ and a high concentration of sparteine with a k_i value of 200 $\mu mol \cdot l^{-1}$, and a significant correlation ($\gamma s = 0.62$, $P < 0.01$) was found between the oxidative activity of reduced haloperidol and sparteine oxidation in a study involving 17 human liver samples.

In the present study, however, it was concluded that CYP3A4, not CYP2D6, was the principal isoform of cytochrome P450 responsible for the metabolic conversion of reduced haloperidol to haloperidol. This conclusion differed from that reported by Tyndale et al. Quinidine is not only a potent inhibitor of CYP2D6 [26–28], but also a substrate of CYP3A4 [29]. Thus, it was considered that the CYP3A4-catalyzed oxidation of reduced haloperidol to haloperidol was inhibited by quinidine with a k_i value of 24.9 $\mu mol \cdot l^{-1}$ in the present study. For the same reason, CYP3A4 might also be involved in the metabolic conversion of reduced bromperidol to bromperidol [24].

There has been no investigation of whether sparteine is a substrate or inhibitor of CYP3A4, whereas the compound is known to be a substrate of CYP2D6 [30, 31]. However, sparteine competitively inhibited the CYP3A4-catalyzed oxidation of reduced haloperidol with a high k_i value of 1390 $\mu mol \cdot l^{-1}$ in the present study. Accordingly, it was considered that a high concentration of sparteine might inhibit CYP3A4 activity.

Carteolol is a nonselective β-adrenoceptor antagonist [32, 33], which is prescribed clinically for the treatment of hypertension and other cardiovascular diseases. Only CYP2D6 could catalyze the reaction of carteolol 8-hydroxylation, and CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2E1, and 3A4 were not involved in carteolol metabolism [25]. The k_M and V_{max} of carteolol 8-hydroxylation by CYP2D6 were 183 $\mu mol \cdot l^{-1}$ and 26.1 $pmol \cdot min^{-1} \cdot pmol^{-1}$ P450, respectively, with the k_M value being higher than that of propranolol (3–6 $\mu mol \cdot l^{-1}$ [34]), nortriptyline (16 $\mu mol \cdot l^{-1}$ [35]), dextromethorphan (5.3 $\mu mol \cdot l^{-1}$ [36]), sparteine (73 $\mu mol \cdot l^{-1}$ [31]), bufuralol (61 $\mu mol \cdot l^{-1}$ [37]), debrisoquine (130 $\mu mol \cdot l^{-1}$ [38], S-(−)-metoprolol (69 $\mu mol \cdot l^{-1}$ [28]), R-(+)-metoprolol (41 $\mu mol \cdot l^{-1}$ [28]), minapril (5.26 $\mu mol \cdot l^{-1}$ [39]), and bupranolol (0.27 $\mu mol \cdot l^{-1}$ [40]), all of which were substrates for CYP2D6 activity. These results indicate that carteolol 8-hydroxylation by CYP2D6 is a selective and sensitive reaction probe for the determination of CYP2D6 activity. Thus, the effects of reduced haloperidol or haloperidol on CYP2D6 activity were investigated using CYP2D6-catalyzed carteolol 8-hydroxylation as a reaction probe for the enzyme activity.

Studies carried out with human liver preparations in vitro have shown both haloperidol and reduced haloperidol to be potent inhibitors of CYP2D6 [19, 41], and investigators have reported that reduced haloperidol was five times more potent than haloperidol in inhibiting sparteine metabolism [19]. Similar results were also shown in the present study using carteolol 8-hydroxylation as a reaction probe for CYP2D6 activity, except that haloperidol was not only an inhibitor of the enzyme, but also a stimulator of the enzyme at an optimum concentration of 1 $\mu mol \cdot l^{-1}$. This is the first study reporting the stimulatory effect of haloperidol on CYP2D6 activity.

The in vitro activation of cytochrome P450-mediated monooxygenase activity by various chemicals, including for acetone, butanone, metyrapone, ethyl isocyanide, detergents, steroids, carcinogens, and naturally occurring and synthetic flavonoids, has been observed on numerous occasions [42–44]. The mechanisms involved in the enhancement of the enzyme activity have been suggested to be as follows. Acetone may effect the peroxidative function of cytochrome P450 or a step beyond cytochrome P450 (45). α-Naphthoflavone exerts its stimulatory effect on cytochrome P450 activity by an allosteric phenomenon [43], and flavonoids exert their stimulatory effect by enhancing the interaction between cytochrome P450 and NADPH cytochrome-P450 reductase, thereby facilitating the flow of electrons to the hemoprotein [42]. However, it seems that the details on

the mechanisms of the in vitro enhancement of drug metabolism by some activators, including the stimulatory effect of haloperidol on CYP2D6 activity, are still unclear. In further investigations concerning the mechanisms of that enhancement, haloperidol, which enhances CYP2D6 activity, would be a useful tool in the elucidation of the catalytic functions of the enzyme.

In conclusion, the combined results obtained from the investigation of various CYP isoforms and of chemical inhibition in the present study demonstrated that the metabolic conversion of reduced haloperidol to haloperidol was mediated by the single isoform CYP3A4, and not by CYP2D6. Recently, Fang et al. reported the same finding on the role of CYP3A4 in the oxidation of reduced haloperidol to haloperidol [46]. Moreover, it was indicated that reduced haloperidol is a substrate of CYP3A4 and an inhibitor of CYP2D6 and that haloperidol has both stimulatory and inhibitory effects against CYP2D6 activity.

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