Bioactivation of Tegafur to 5-Fluorouracil Is Catalyzed by Cytochrome P-450 2A6 in Human Liver Microsomes *in Vitro*[1]

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ABSTRACT

Tegafur is a prodrug of 5-fluorouracil (5-FU) consisting of a new class of oral chemotherapeutic agents, tegafur/uracil and S-1, which are classified as dihydropyrimidine dehydrogenase inhibitory fluoropyrimidines. It is bioactivated to 5-FU via 5′-hydroxylation mediated by cytochrome P-450 (CYP). However, which isoform(s) of CYP is responsible for the bioactivation process of tegafur remains unclear. The purpose of the present study was to identify the human CYP isoform(s) involved in the metabolic activation of tegafur using human liver microsomes and cDNA-expressed human CYPs.

The formation of 5-FU from tegafur in human liver microsomes showed biphase kinetics with K_m and V_{max} values for the high-affinity component of 0.43 ± 0.05 mM and 4.02 ± 1.70 nmol/mg/min (mean ± SD, $n = 4$), respectively. In the correlation study using a panel of 10 human liver microsomes, the formation of 5-FU from tegafur showed a significant correlation ($r = 0.98$; $P < 0.001$) with coumarin 7-hydroxylation, a marker activity of CYP2A6. In addition, a specific substrate of CYP2A6 and anti-CYP2A6 antibody inhibited the formation of 5-FU by 90% in human liver microsomes. Moreover, cDNA-expressed CYP2A6 showed the highest activity for the formation of 5-FU among 10 cDNA-expressed CYPs, with a K_m value similar to that found for the high-affinity component in human liver microsomes.

These findings clearly suggest that CYP2A6 is a principal enzyme responsible for the bioactivation process of tegafur in human liver microsomes. However, to what extent the bioactivation of tegafur by CYP2A6 accounts for the formation of 5-FU *in vivo* remains unclear, because the formation of 5-FU from tegafur is also catalyzed by the soluble fraction of a 100,000 × *g* supernatant and also derived from spontaneous degradation of tegafur.

INTRODUCTION

Tegafur is a prodrug of 5-FU[3] synthesized >30 years ago (1). Although its development was abandoned in the United States for more than two decades, it has been developed as a new class of oral chemotherapeutic agent in Japan. These new agents include tegafur/uracil and S-1, which are known as dihydropyrimidine dehydrogenase inhibitory fluoropyrimidines (2, 3). They have been so classified because they are composed of 5-FU prodrug and uracil or 5-chloro-2,4-dihydroxypyridine, which prevents the degradation of 5-FU, thus ensuring that the concentration of 5-FU remains at sustained levels in both the plasma and the tumor (3, 4). Tegafur/uracil and S-1 have been commercially available in Japan since 1983 and 1999, respectively, and have been extensively studied for their effectiveness in treating various tumors, including colon rectal cancer (3), gastric carcinoma (5), pulmonary malignancy (6), and head and neck cancer (7). Recently, they have been subjected to clinical evaluation in the United States (2–7).

Tegafur is a depot form of fluorouracil, which releases 5-FU slowly in the body. The conversion of tegafur to fluorouracil mainly occurs in the liver. As illustrated in Fig. 1, it is hydroxylated at the 5′-position of a furan ring to produce 5′-hydroxytegafur, which is unstable and is spontaneously decomposed to 5-FU (8). This bioactivation process is thought to be mediated by CYP, because NADPH is required for the conversion of tegafur to 5-FU in liver microsomes (9), and its conversion rate has been shown to be accelerated by the pretreatment of mice with phenobarbital (10), a well-known inducer of CYP. However, which isoform(s) of CYP is responsible for the bioactivation process of tegafur remains unclear.

Identification of the human isoform of CYP responsible for the metabolism of a drug is useful for assessing the interindividual variability in its metabolism, particularly when the isoform of CYP exhibits genetic polymorphism (11). In addition, there are data available for a number of compounds that inhibit

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[3] The abbreviations used are: 5-FU, 5-fluorouracil; CYP, cytochrome P-450; OR, NADPH cytochrome P-450 oxidoreductase.

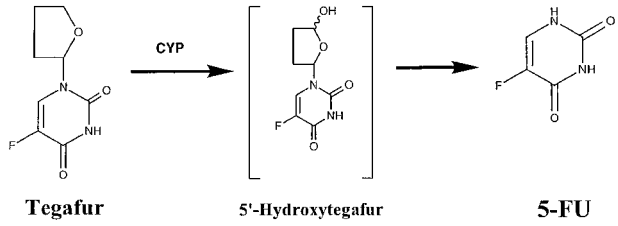

Fig. 1 Proposed metabolic pathways of tegafur to 5-FU via CYP. Tegafur is hydroxylated by CYP to 5′-hydroxytegafur, which is unstable and spontaneously degraded to 5-FU and a succinic aldehyde moiety (8).

or induce specific isoforms of CYP (12). Therefore, it will be possible to estimate the variability of metabolism or drug interactions when the specific isoform of CYP responsible for the bioactivation of tegafur in humans is identified. In this study, we identified a human CYP isoform involved in the metabolic activation of tegafur using human liver microsomes and cDNA-expressed human CYPs.

MATERIALS AND METHODS

Chemicals. Tegafur and 5-chloro-2,4-dihydroxypyridine were obtained from Taiho Pharmaceutical Co. (Saitama, Japan). $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase, α-naphthoflavone, quinidine, sulfaphenazole, and troleandomycin were purchased from Sigma Chemical Co. (St. Louis, MO). Coumarin and *p*-nitrophenol were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other chemicals were of the highest grade commercially available.

Enzymes and Antibodies. Human liver microsomes were obtained from the International Institute for the Advancement of Medicine (Exton, PA) or SRI International (Menlo Park, CA). The human liver microsomes were diluted with 100 mM Tris-HCl buffer (pH 7.4) containing 1 mM DTT and 20% (v/v) glycerol and stored at −80°C until use. Microsomes prepared from human B-lymphoblastoid cells expressing CYP1A1 + OR, CYP1A2, CYP2A6 + OR, CYP2B6, CYP2C8 + OR, CYP2C9(Arg) + OR, CYP2C19, CYP2D6(Val) + OR, CYP2E1 + OR, and CYP3A4 + OR were obtained from Gentest (Woburn, MA). Control microsomes expressed only the vector. A monoclonal antibody against human CYP2A6 was obtained from Gentest.

Assay with Human Liver Microsomes. The basic incubation mixture contained 0.5 mg/ml of human liver microsomes, 100 mM Tris-HCl buffer (pH 7.4), a NADPH-generating system (2 mM $NADP^+$, 5 mM glucose-6-phosphate, 1 IU/ml of glucose-6-phosphate dehydrogenase, and 5 mM $MgCl_2$), 0.4 mM 5-chloro-2,4-dihydroxypyridine, and 1 mM tegafur in a final volume of 250 or 500 μl. The mixture was incubated at 37°C for 10 min. After the reaction was stopped by acute freezing in acetone containing dry ice, the mixture was stored at −80°C until analysis. The formation of 5-FU was determined by gas chromatography/mass spectrometry (13). Because 5-FU is extensively metabolized by a cytosolic protein, dihydropyridine dehydrogenase, the amount of 5-FU formed from tegafur in human liver microsomes is decreased by contaminated dihydropyridine dehydrogenase. Therefore, 0.4 mM 5-chloro-2,4-dihydroxypyridine was added in the incubation mixture as a potent inhibitor of dihydropyridine dehydrogenase (14). This concentration of 5-chloro-2,4-dihydroxypyridine did not inhibit the marker activities of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A in human liver microsomes (data not shown).

Kinetic Studies. Kinetic studies were performed using microsomes from four human livers (H-33, H-35m H-69, and HHM0071). In determining kinetic parameters, the tegafur concentration ranged from 0.5–20 mM. All reactions were performed in a linear range with respect to protein concentration and incubation time, 0.5 mg/ml microsomal protein and 10-min incubation time. Because tegafur is nonenzymatically converted to 5-FU, the content of 5-FU in the mixture incubated without microsomal protein was subtracted from that with microsomal protein to correct the activity. Eadie-Hofstee plots were constructed for determination of the presence of a mono- or biphasic model. Because reactions followed biphasic Michaelis-Menten kinetic behavior (*i.e.*, two-enzyme kinetics), Michaelis-Menten kinetic parameters were estimated by fitting the data to the following equation (15):

$$V = V_{max}1 \times S/(K_m1 + S) + V_{max}2 \times S/(K_m2 + S) \quad (A)$$

where V is the formation rate of 5-FU, S is the concentration of tegafur in the incubation mixture, K_m1 and K_m2 are the affinity constants for the high- and low-affinity components, respectively, and $V_{max}1$ and $V_{max}2$ are the maximum enzyme velocities for the high- and low-affinity components, respectively. Kinetic parameters were estimated initially by graphic analysis of Eadie-Hofstee plots, and the values were subsequently used as initial estimates for nonlinear regression analysis using a computer program (DeltaGraph program; Statistical Product and Service Solutions, Inc., Chicago, IL). When the data did not fit Eq. A, kinetic parameters of the reaction following biphasic Michaelis-Menten kinetic behavior were estimated by fitting the data to the following equation (16):

$$V = V_{max}1 \times S/(K_m1 + S) + L \times S \quad (B)$$

where L represents the intrinsic clearance or V_{max}/K_m of low-affinity enzyme (*i.e.*, $V_{max}2/K_m2$). Because reactions followed a simple Michaelis-Menten kinetic behavior (*i.e.*, one-enzyme kinetics), the kinetic parameters (K_m, V_{max}, and V_{max}/K_m) of recombinant CYPs were estimated by nonlinear least-squares regression analysis.

Correlation Study. Correlation between the formation rate of 5-FU at a 1-mM substrate concentration and 7-ethoxyresorufin *O*-deethylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), 7-benzyloxyresorufin *O*-debenzylase (CYP2B6), tolbutamide methylhydroxylase (CYP2C9), *S*-mephenytoin 4′-hydroxylase (CYP2C19), dextromethorphan *O*-demethylase (CYP2D6), *p*-nitrophenol hydroxylase (CYP2E1), and testosterone 6β-hydroxylase (CYP3A) activities were studied by using microsomes from 10 human livers. The isoform-specific activities were determined as described previously (17–26). The correlation coefficient (r) was calculated by the least-squares regression method using an SAS system for Windows (version 6.12; SAS Institute, Inc.).

Inhibition Study. The effects of CYP isoform-specific inhibitors or substrates (*i.e.*, compounds able to act as compet-

itive inhibitors) on the formation of 5-FU at a 1 mM substrate concentration were investigated using microsomal preparations obtained from a human liver specimen (HHM-0071). The inhibitors used in this part of the study were α-naphthoflavone (a CYP1A2 inhibitor; Ref. 27), coumarin (a CYP2A6 substrate; Ref. 19), sulfaphenazole (a CYP2C8/9 inhibitor; Ref. 27), quinidine (a CYP2D6 inhibitor; Ref. 27), *p*-nitrophenol (a CYP2E1 inhibitor; Ref. 28), and troleandomycin (a CYP3A inhibitor; Ref. 27). The range of concentration used was 1–100 μM. Inhibitors were dissolved in DMSO and diluted with 100 mM Tris-HCl buffer (pH 7.4) so that the final concentration of solvent in the incubation mixture was 0.5%. The same concentration of DMSO was also added to the control. The incubation mixture, including chemical inhibitors, was preincubated for 5 min before the reaction was initiated by the addition of a substrate.

Inhibitory effects of antibodies to human CYP2A6 were examined by preincubating microsomes with the antibodies for 30 min on ice. Tegafur (1 mM) and other components of the incubation medium were then added, and the reaction was carried out as described above. The amounts of monoclonal antibodies against human CYP2A6 used were up to 0.8 μg/μg microsomal protein. This monoclonal antibody is a potent CYP2A6-specific inhibitor yielding 90% inhibition of human liver microsomal CYP2A6, whereas it does not inhibit human CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.[4]

Assay with Recombinant CYPs. Microsomes from human B-lymphoblastoid cells expressing CYP1A1 (lot 19), CYP1A2 (lot 43), CYP2A6 (lot 26), CYP2B6 (lot 35), CYP2C8 (lot 13), CYP2C9 (lot 7), CYP2C19 (lot 9), CYP2D6 (lot 11), CYP2E1 (lot 26), and CYP3A4 (lot 31) were used. The reactions were carried out as described for the human liver microsomal study. To examine the role of individual CYP isoforms involved in the formation of 5-FU from tegafur, each of the recombinant CYPs (0.5 mg/ml) described above was incubated with 1 mM tegafur for 10 min at 37°C, according to the procedure recommended by the supplier.

Kinetic studies were performed using microsomes from human B-lymphoblastoid cells expressing CYP2A6 and CYP2C9. In determining kinetic parameters, the tegafur concentration ranged from 0.5 to 20 mM. All reactions were performed in a linear range with respect to protein concentration and incubation time, 0.5 mg/ml microsomal protein and 10-min incubation time. The kinetic parameters were estimated as described above.

RESULTS

Kinetics of 5-FU Formation from Tegafur in Human Liver Microsomes. Eadie-Hofstee plots for the formation of 5-FU from tegafur (0.5–20 mM) in four human liver microsomes are shown in Fig. 2. The plots showed biphasic curves, suggesting that the reaction showed multiple-enzyme kinetic behavior. Because the data obtained from three human liver microsomes

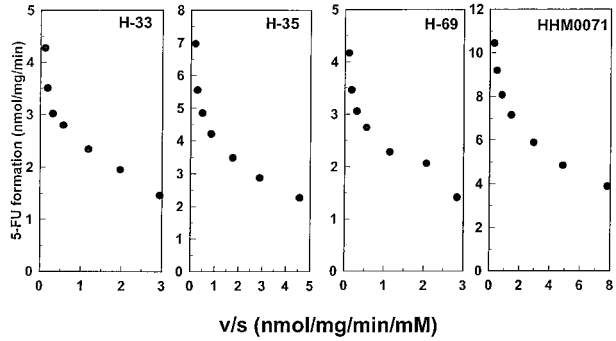

Fig. 2 Eadie-Hofstee plots of 5-FU formation from tegafur in microsomal samples from four human livers (H-33, H-35, H-69, and HHM-0071). *v*, velocity of metabolite formation; *s*, substrate concentration. Tegafur (0.5–20 mM) was incubated at 37°C for 10 min with 0.5 mg/ml of human liver microsomes. Each data point represents the mean of duplicate experiments.

(H-35, H-69, and HHM-0071) fitted Eq. A, the kinetic parameters for high- and low-affinity components were estimated. For a microsomal sample (H-33), the kinetic parameters for the high-affinity component were estimated by fitting the data to Eq. B. The kinetic parameters for the formation of 5-FU in four human liver microsomes are listed in Table 1. The mean (± SD) kinetic parameters for the high-affinity component obtained from four human liver microsomes were: $K_m1 = 0.43 \pm 0.05$ mM, and $V_{max}1 = 4.02 \pm 1.70$ nmol/mg/min. The $V_{max}1/K_m1$ value was 88-fold greater that the $V_{max}2/K_m2$ value (9.74 ± 5.42 *versus* 0.11 ± 0.11 μl/mg/min).

Correlation Study. The formation rate of 5-FU from tegafur in human liver microsomes varied 40-fold (0.12–5.01 nmol/mg/min) with the median value of 1.01 nmol/min/mg among the 10 samples studied. The formation rate of 5-FU from tegafur and the CYP isoform-selective catalytic activity in the same panel of 10 human liver microsomes were compared. As shown in Fig. 3, the formation rate of 5-FU in the 10 human liver microsome preparations at 1 mM tegafur was significantly correlated with coumarin 7-hydroxylase activity ($r = 0.98$; $P < 0.001$). No other significant correlations were observed between the formation rate of 5-FU and catalytic activities of 7-ethoxyresorufin *O*-deethylase ($r = 0.38$; $P = 0.277$), 7-benzyloxyresorufin *O*-debenzylase ($r = 0.12$; $P = 0.747$), tolbutamide methylhydroxylase ($r = 0.52$; $P = 0.116$), *S*-mephenytoin 4′-hydroxylase ($r = 0.37$; $P = 0.297$), dextromethorphan *O*-demethylase ($r = 0.10$; $P = 0.783$), *p*-nitrophenol hydroxylase ($r = 0.41$; $P = 0.244$), or testosterone 6β-hydroxylase ($r = 0.45$; $P = 0.200$).

Inhibition Study. CYP isoform-specific xenobiotic compounds were screened for inhibitory effects on the formation of 5-FU from tegafur in human liver microsomes (Fig. 4). Coumarin, a specific substrate of CYP2A6, inhibited the formation of 5-FU in human liver microsomes in a concentration-dependent manner. The formation of 5-FU was inhibited by α-naphthoflavone and *p*-nitrophenol, whereas the extent of inhibition by these inhibitors was slight (<20%), even at a concentration of 100 μM. No effects of sulfaphenazole, quinidine, and troleandomycin were observed with inhibitor concentrations up to 100 μM.

[4] Gentest (Woburn, MA), unpublished data.

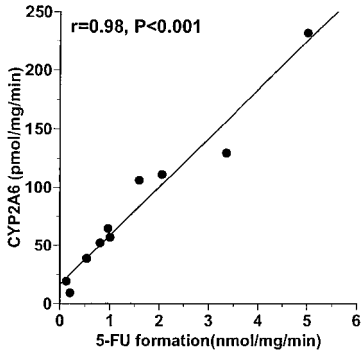

Fig. 3 Correlation between 5-FU formation and CYP2A6 activity in 10 human liver microsomes. The formation rates of 5-FU from tegafur in 10 human liver microsomes were compared with the coumarin 7-hydroxylase activity catalyzed by CYP2A6. Tegafur (1 mM) was incubated at 37°C for 10 min with 0.5 mg/ml of human liver microsomes. Coumarin (20 μM) was incubated at 37°C for 10 min with 0.4 mg/ml of human liver microsomes. The correlation coefficient (*r*) was calculated by the least-squares regression method.

Similar results were obtained in the antibody inhibition study. As shown in Fig. 5, anti-CYP2A6 antibodies showed 90% inhibition against the formation of 5-FU from tegafur at 0.8 μg/μg microsomal protein.

Study with cDNA-expressed CYPs. Microsomes from human B-lymphoblastoid cells expressing CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were examined in terms of the abilities of individual CYP proteins to catalyze the formation of 5-FU from tegafur. As shown in Fig. 6, formation of 5-FU was found in cDNA-expressed CYP2A6 and CYP2C9 (7.14 and 1.67 pmol/pmol CYP/min, respectively). Other isoforms showed negligible activity for the formation of 5-FU from tegafur. Accordingly, kinetic analysis for the formation of 5-FU was performed using the microsomes from cDNA-expressed CYP2A6 and CYP2C9. Because Eadie-Hofstee plots for the formation of 5-FU in cDNA-expressed CYP2A6 and CYP2C9 were linear (data not shown), kinetic parameters were estimated by nonlinear least-squares regression analysis. The apparent K_m of CYP2A6 (0.53 mM) was >20-fold lower than that of CYP2C9 (12 mM). The V_{max} values of CYP2A6 and CYP2C9 were 38.54 and 1.56 pmol/pmol CYP/min, respectively. The cDNA-expressed CYP2A6 showed much greater values of V_{max}/K_m than did the cDNA-expressed CYP2C9 (72.4 *versus* 0.1 μl/nmol CYP/min).

DISCUSSION

The results of the present study clearly showed that CYP2A6 is a principal enzyme responsible for the bioactivation process of tegafur in human liver microsomes. The supporting evidence can be summarized as follows: (*a*) the formation rate of 5-FU from tegafur in a panel of 10 human liver microsomes was significantly correlated with coumarin 7-hydroxylase activity, a marker activity of CYP2A6 ($r = 0.98$; $P < 0.001$; Fig. 3), but it was not correlated with other activities of CYPs; (*b*) coumarin, a specific substrate of CYP2A6, inhibited the formation of 5-FU in human liver microsomes in a concentration-dependent manner (Fig. 4); (*c*) the anti-CYP2A6 antibody inhibited the formation of 5-FU by 90% in human liver microsomes (Fig. 5); (*d*) cDNA-expressed CYP2A6 showed the highest activity for the formation of 5-FU among the 10 cDNA-expressed CYPs (Fig. 6) with a K_m similar to that found for the high-affinity component in human liver microsomes (Table 1). Although cDNA-expressed CYP2C9 showed the second-highest activity for the formation of 5-FU from tegafur (Fig. 6), it does not appear to contribute significantly to the bioactivation of tegafur in human liver microsomes, because the intrinsic clearance (V_{max}/K_m) for the formation of 5-FU from tegafur was much smaller than that of CYP2A6.

CYP2A6 is an enzyme showing genetic polymorphism (29). To date, there have been four mutant alleles, designated as *CYP2A6*2, *3, *4,* and **5,* in addition to the wild-type allele (*CYP2A6*1*; Ref. 30). *CYP2A6*2* has a single base mutation (T→A), leading to a change from Leu-160 to His-160 in exon 3, which results in the formation of a catalytically inactive enzyme (31). *CYP2A6*3* has been proposed to be inactive, because it was formed by the gene conversion of *CYP2A6*1* and functionally inactive *CYP2A7* gene in exons 3, 6, and 8 (31), although its functionality is not fully understood because of the low prevalence of homozygotes for this mutant allele. The gene frequencies of *CYP2A6*2* are 1.1, 1.4, 3.0, and 2.3% in Swedes, Finns, Spaniards (32), and Germans (33), respectively, and that of *CYP2A6*3* is 1.4% in Germans (33), but they have not been detected in Japanese (34). *CYP2A6*4* is an allele with a partial or whole deletion of the *CYP2A6* gene (35–38). Microsomes prepared from the liver of a homozygote for the gene deletion of *CYP2A6* are devoid of CYP2A6 protein (29, 35). This type of mutant gene is relatively common in Oriental populations, with frequencies of 15.1% in Chinese (37) and 4.5% in Japanese (38), whereas it is rare in Finns (1.0%) and Spaniards (0.5%; Ref. 37). *CYP2A6*5* is a new defective allele, but it is rare in Chinese (1.0%) and has not been detected in Spaniards (30).

These mutant alleles of CYP2A6 have been reported to be associated with decreased metabolic clearances of several drugs or environmental chemicals. For example, the metabolic clearance of SM-12502, a platelet-activating factor receptor antagonist, which is entirely metabolized by CYP2A6, was shown to be markedly decreased in subjects with a *CYP2D6* gene deletion (36). Similarly, the formation of cotinine from nicotine, which is predominantly catalyzed by CYP2A6, is dramatically decreased in homozygous subjects with a *CYP2A6* gene deletion (39). These findings coupled with the present observations suggest that bioactivation of tegafur is suppressed and that there is an insufficient level of 5-FU in the plasma or tumor of patients treated with tegafur/uracil or S-1 if they carry mutant alleles of *CYP2A6*. However, to what extent the bioactivation of tegafur by CYP2A6 accounts for the formation of 5-FU *in vivo* remains unclear, because the K_m for the formation of 5-FU from tegafur obtained in the present study (*i.e.*, 0.43 mM; Table 1) is higher than the plasma concentrations of tegafur (0.02–0.05 mM) after the repeated administration of tegafur/uracil to the patients, 300 mg/m^2/day (40). In addition, the formation of 5-FU from tegafur is also catalyzed by the soluble fraction of a 100,000 × *g* supernatant, and 5-FU is derived from

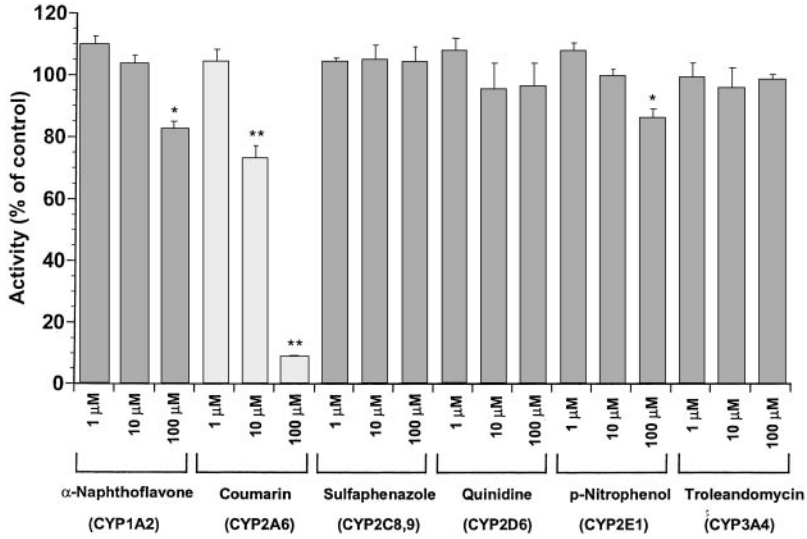

Fig. 4 Effects of chemical inhibitors for CYPs on the formation of 5-FU from tegafur in human liver microsomes. Tegafur (1 mM) was incubated at 37°C for 10 min with 0.5 mg/ml of human liver microsomes (HHM-0071). Inhibitors were dissolved in DMSO and added to the incubation mixture at a final acetonitrile concentration of <5%. Human liver microsomes (0.5 mg/ml) were preincubated with various concentrations of inhibitors for 5 min at 37°C before the addition of a substrate. Data are expressed as percentages of the formation rate of 5-FU in the incubation mixture containing the same concentration of solvent without inhibitors. Each *column* represents the mean of three different experiments; *bars*, SD. *, $P < 0.01$; **, $P < 0.001$.

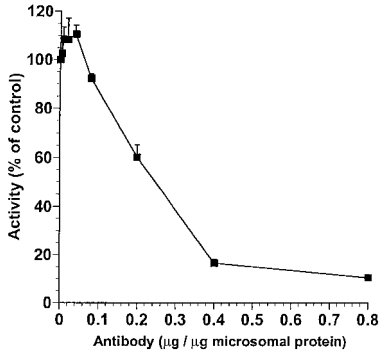

Fig. 5 Effects of anti-CYP2A6 antibodies on the formation of 5-FU from tegafur in human liver microsomes (HHM-0071). Human liver microsomes (0.5 mg/ml) were preincubated with various amounts of anti-CYP2A6 antibodies (0 to 0.8 mg/mg microsomal protein) for 30 min on ice. The incubation medium containing tegafur (1 mM) and other components were added to the mixture of microsomes and antibodies, and the reaction was carried out as described in "Materials and Methods." Data are expressed as percentages of the formation of 5-FU in the absence of antibodies. Each *point* represents the mean of three different experiments; *bars*, SD.

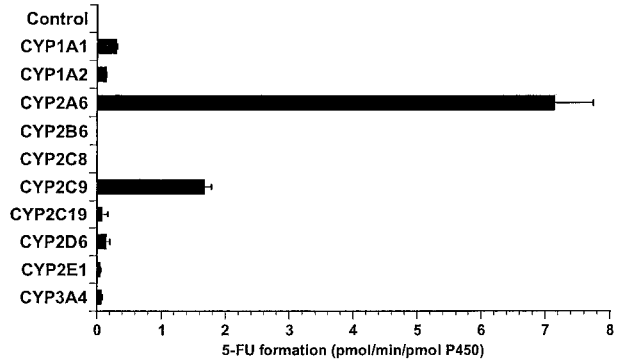

Fig. 6 Formation rate of 5-FU from tegafur in microsomes from human B-lymphoblastoid cells expressing individual human CYPs. A substrate (1 mM tegafur) was incubated at 37°C for 10 min with microsomes (0.5 mg/ml) from human B-lymphoblastoid cells expressing CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Each *column* represents the mean of three different experiments; *bars*, SD.

spontaneous degradation of tegafur (41, 42). Therefore, further *in vivo* studies are clearly needed to clarify the influence of CYP2A6 polymorphism on the formation of 5-FU in patients taking tegafur/uracil or S-1.

Because few drugs are known to be metabolized by CYP2A6, there is limited information on drug interaction caused by the inhibition or induction of CYP2A6. Mäenpä *et al*. (43) reported that methoxalen, an antipsoriatic agent, inhibits the formation of 7-hydroxycoumarin from coumarin, the activity of which is the marker activity of CYP2A6 *in vivo* in human volunteers. Methoxalen was reported to be a mechanism-based inhibitor of CYP2A6 (44). It was also reported that grapefruit juice flavonoids inhibit the formation of 7-hydroxycoumarin in healthy volunteers (45). These findings suggest that the formation of 5-FU from tegafur may be suppressed by the concurrent administration of methoxalen or by taking tegafur with grapefruit juice. On the other hand, patients treated with antiepileptic drugs excrete all of the measurable 7-hydroxycoumarin in the first 4 h (46), suggesting that CYP2A6 is inducible (29). In accordance with the *in vivo* findings, an *in vitro* study using primary human hepatocytes indicated that CYP2A6 is induced by treatment with phenobarbital or rifampicin (29). These findings suggest that the formation of 5-FU from tegafur is accelerated in patients treated with antiepileptic drugs or rifampicin.

In conclusion, the results of the present study clearly showed that CYP2A6 is a principal enzyme responsible for the bioactivation process of tegafur in human liver microsomes. This suggests that genetic polymorphism of CYP2A6 and/or drug interaction affect the formation of 5-FU from tegafur and modify the outcome of cancer chemotherapy using tegafur/

Table 1 Kinetic parameters of 5-FU formation from tegafur in microsomes from four human livers

	High-affinity			Low-affinity		
Human liver	K_m1 mM	$V_{max}1$ nmol/mg/min	$V_{max}1/K_m1$ μl/mg/min	K_m2 mM	$V_{max}2$ nmol/mg/min	$V_{max}2/K_m2$ μl/mg/min
H-33	0.47	2.81	6.02			0.04
H-35	0.44	4.09	9.32	166	14.99	0.09
H-69	0.46	2.79	6.09	374	14.58	0.04
HHM-0071	0.37	6.40	17.54	24	6.56	0.27
Mean	0.43	4.02	9.74			0.11
SD	0.05	1.70	5.42			0.11

uracil or S1, although further studies are clearly needed to confirm these possibilities.

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