Involvement of Microsomal Cytochrome P450 and Cytosolic Thymidine Phosphorylase in 5-Fluorouracil Formation from Tegafur in Human Liver

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ABSTRACT

Recently, we have reported that tegafur, an anticancer agent, is biotransformed into active drug 5-fluorouracil (5-FU) by cytochromes P450 1A2, 2A6, and 2C8 in human liver microsomes (T. Komatsu et al., Drug Metab. Dispos., 28: 1457–1463, 2000). Because the conversion of tegafur into 5-FU has also been reported to be catalyzed by cytosolic thymidine phosphorylase (dThdPase), the involvement of human liver microsomes and cytosol and individual differences in 5-FU formation from tegafur were investigated. In 14 human samples, the mean rates of 5-FU formation in liver microsomes and cytosol showed 5- and 3-fold interindividual differences at 100 μM and 1 mM tegafur, respectively. In the presence of 5-chloro-2,4-dihydroxypyridine, a dihydropyrimidine dehydrogenase inhibitor, the rates of 5-FU formation by the combination of liver microsomes and cytosol showed 5- and 3-fold interindividual differences at 100 μM and 1 mM tegafur, respectively. Kinetic analysis of human liver cytosolic 5-FU formation indicated an apparent lower Kₘ value (16 ± 4 mM) than that of liver microsomes (1.8 ± 0.3 mM) with similar Vₘax values. Human liver cytosolic 5-FU formation was confirmed to be catalyzed by dThdPase with correlation and chemical inhibition studies. These results suggested that 5-FU formation from tegafur in human liver was mainly catalyzed by microsomal P450 at low concentrations of tegafur, but the contribution of cytosolic 5-FU formation by dThdPase would be important at high concentrations.

INTRODUCTION

Tegafur [5-fluoro-1-(2-tetrahydrofuryl)-2,4(1H, 3H)-pyrimidinedione], a prodrug of 5-FU, has been clinically used for over 20 years as an anticancer drug (1). It has been reported that tegafur is converted into 5-FU mainly in the liver and that 5-FU may inhibit the growth of cancer cells by the inhibition of thymidylate synthase or by its incorporation into RNA (2, 3). 5-FU is further biotransformed into an inactive molecule by DPD in the liver cytosol (3).

It has been considered that there are two pathways in the conversion of tegafur into 5-FU, mainly in the microsomal fraction and partly in the cytosolic fraction (4–6). It has been reported that liver microsomal 5-FU formation from tegafur is catalyzed by P450 (6, 7). However, the precise roles of P450 have still not been clarified. Recently, we reported that human CYP1A2, CYP2A6, and CYP2C8 have different contributions to the biotransformation of tegafur into 5-FU in individual human liver microsomes (8). On the other hand, cytosolic 5-FU formation from tegafur is thought to be catalyzed by dThdPase (9, 10). dThdPase, purified from human gastric tumor tissues, has been shown to catalyze 5-FU formation from tegafur as well as thymine formation from thymidine (10). The levels of dThdPase were reported to be higher in various tumor tissues than in normal tissues (11, 12). Therefore, 5-FU formation from tegafur in tumor tissues is considered to be mainly catalyzed by dThdPase. However, the involvement of microsomal P450 and cytosolic dThdPase in 5-FU formation from tegafur in human liver or tumor tissues has not been evaluated and interindividual differences in microsomal and cytosolic 5-FU formation have not been examined in detail.

In the present study, we investigated the involvement of human liver microsomes and cytosol in 5-FU formation from tegafur. Interindividual differences in 5-FU formation by the combination of liver microsomes and cytosol were determined in the presence of 5-chloro-2,4-dihydroxypyridine, a DPD inhibitor. Moreover, we also confirmed that cytosolic 5-FU formation was mainly catalyzed by dThdPase, not by UrdPase.

MATERIALS AND METHODS

Chemicals. Tegafur (>99.9%), trans-4’-hydroxy-tegafur, cis-4’-hydroxy-tegafur, trans-3’-hydroxy-tegafur, cis-3’-hydroxy-tegafur, 4’-5-dehydro-tegafur, 5-chloro-2,4-dihydroxypyridine, and TPI were synthesized by Taiho Pharmaceutical.

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The abbreviations used are: 5-FU, 5-fluorouracil; 5’-dFUrd, 5’-deoxy-5-fluorouridine (doxifluridine); DPD, dihydropyrimidine dehydrogenase; dThdPase, thymidine phosphorylase; P450, general term for cytochrome P450; CYP, individual forms of P450; S9, 9000 × g supernatant; TPI, 5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4(1H,3H)-pyrimidine-dinedione; UrdPase, uridine phosphorylase; HPLC, high-performance liquid chromatography.
Tokushima, Japan. 5′-dFUrld was provided by Nippon Roche, Tokyo, Japan. 5′-dFUrld is also a produg of 5-FU and has been known to be biotransformed into 5-FU by dThdPase (10, 13). 5-FU and 2,6-dihydroxypyridine were obtained from Wako Pure Chemicals (Osaka, Japan) and Aldrich (Milwaukee, WI), respectively. 5-Bromouracil, 5-chlorouracil, 6-benzyl-2-thiouracil, thymine, thymidine, uracil, and uridine were obtained from Sigma (St. Louis, MO). Other chemicals used in this study were obtained from sources described previously (8) or were of the highest quality commercially available.

**Enzyme Preparations.** Human livers (HL-1, 3, 4, 5, 6, 9, and 10; Refs. 14, 15) were homogenized in three volumes of 0.1 M Tris-HCl buffer (pH 7.4) containing 0.1 mM EDTA and 0.1 mM KCl, and the homogenate was centrifuged at 9,000 × g for 15 min. A portion of the supernatant was used as the human liver S9 fraction. The supernatant was further centrifuged at 105,000 × g for 90 min. The 105,000-g supernatant was dialyzed using cellulose tubing (Spectrum Laboratories, Laguna Hills, CA) overnight against 200 volumes of 20 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM β-mercaptoethanol according to the methods described previously (16) and was used as the human liver cytosol. Human liver microsomes were prepared in 10 mM Tris-HCl buffer (pH 7.4) containing 0.10 mM EDTA and 20% (v/v) glycerol as described previously (14). The other human liver microsomes and cytosol (HG3, 23, 42, 56, 70, 89, and 93) were obtained from Gentest (Woburn, MA), and these cytosolic fractions were also dialyzed as described above. dThdPase from *Escherichia coli* (Sigma) and recombinant human dThdPase (human PD-ECGF; R&D Systems Inc., Minneapolis, MN; Ref. 17) were purchased.

**Assay of 5-FU Formation.** The 5-FU formation activities were determined according to methods described previously (8) with slight modifications. The standard incubation mixture (final volume of 0.25 ml) contained human liver microsomes (0.5 mg/ml) or a combination of liver microsomes (0.5 mg/ml) and cytosol (2.5 mg/ml) or S9 (3.0 mg/ml) 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system consisting of 0.5 mM NADP+, 5 mM glucose 6-phosphate, and 0.5 unit/ml glucose 6-phosphate dehydrogenase, and tegafur (100 μM or 1.0 mM). When the combination of liver microsomes and cytosol or S9 was used, 5-chloro-2,4-dihydroxypyridine (100 μM) was added to the incubation mixture to inhibit the cytosolic DPD activity. Human liver cytosol (2.5 mg/ml) or dThdPase (0.01 mg/ml) was used as an enzyme source in the absence of the NADPH-generating system. In some cases, chemical inhibitors dissolved in methanol, except for TP1 in water, were used. The final concentration of organic solvent in the incubation mixture was <1.0%. Incubations were carried out at 37°C for 30 min and terminated by adding 1.5 ml of ethyl acetate and 25 μl of 3 M NaCl. 5-Bromouracil (2 μM) was added as an internal standard. The reaction mixture was extracted twice with ethyl acetate. After centrifugation at 900 × g for 10 min, the organic phase was evaporated to dryness under a gentle N2 stream. The residue was dissolved in 20 mM NaClO4 (pH 2.5). The product formation was determined by HPLC with a C18 (5-μm) analytical column [150 × 4.6 (i.d.) mm, Mightysil RP-18 Aqua; Kanto Chemical, Tokyo, Japan] at 35°C. Elution was conducted by 20 mM NaClO4 (pH 2.5) at a flow rate of 0.8 ml/min. The eluent was monitored at 270 nm and recorded with a Chromatopak C-R3A integrator (Shimadzu, Kyoto, Japan) and noise-base clean Uni-3 (Union, Takasaki, Japan). Retention times were as follows: 5-FU, 4.8 min; 5-bromouracil, 11.3 min; tegafur, 65 min. 5-FU was quantified by comparison with the standard curve using the HPLC peak height ratio to 5-bromouracil. Because a trace peak corresponding to 5-FU (~0.01% of the substrate) was observed in the absence of enzymes, the background levels were subtracted in the calculation of enzymatic activities for 5-FU formation at each substrate concentration.

**Other Assays.** 5-FU formation from 5′-dFUrld was determined by the same method as described above using 1 mM 5′-dFUrld as a substrate. Thymine formation from thymidine and uracil formation from uridine were determined according to the methods reported previously (18) with slight modifications. Briefly, human liver cytosol (0.5 mg/ml) or dThdPase (0.01 mg/ml) was incubated at 37°C for 10 min with 1 mM thymidine or uridine in 0.1 mM potassium phosphate buffer (pH 7.4). The reaction (total 0.2 ml) was terminated by adding 0.1 ml of 10% perchloric acid. After centrifugation at 900 × g for 10 min, 0.5 mM Na2HPO4 (0.1 ml) was added to a portion of the supernatant (0.1 ml). The thymine formed was measured by HPLC with a C18 (5 μm) analytical column [150 × 4.6 (i.d.) mm, Mightysil RP-18; Kanto Chemical]. The elution was conducted with a mixture of 9% methanol/0.1 mM potassium phosphate buffer (pH 7.4) at a flow rate of 1.2 ml/min, and the eluate was monitored at 265 nm. Thymine and thymidine were detected at 2.7 min and 4.6 min, respectively. The uracil formed was measured by
HPLC with a C18 (5 μm) analytical column [150 × 4.6 (i.d.) mm, Mightysil RP-18 Aqua]. The elution was conducted with 0.1 M potassium phosphate buffer (pH 7.4) at a flow rate of 0.6 ml/min, and the eluate was monitored at 265 nm. Uracil and uridine were detected at 5.4 min and 11.3 min, respectively. Thymine and uracil were quantified by comparing the HPLC peak heights to those of authentic standards.

The kinetic analysis of 5-FU formation (0.10–2.0 mM tegafur) was performed using a Kaleida-Graph program (Synergy Software, Reading, PA) designed for nonlinear regression analysis. The correlations between 5-FU formation and thymine or uracil formation in human liver cytosol were analyzed using a linear regression analysis program (Instat program from Graphpad Software).

RESULTS

HPLC Analysis of 5-FU Formation Catalyzed by Human Liver Microsomes, Cytosol, and S9. 5-FU formation from tegafur was determined by HPLC, and typical HPLC chromatograms are shown in Fig. 1. After the incubation of tegafur with human liver microsomes (a sample HL-4, 0.5 mg/ml) in the presence of an NADPH-generating system, the formation of 5-FU was observed (Fig. 1A). 5-FU were also formed by human liver cytosol (2.5 mg/ml) in the absence of the NADPH-generating system (Fig. 1B). The rate of 5-FU formation by cytosol (per min) was as high as that of microsomal 5-FU formation in human sample HL-4. Because liver S9 contained ~5-fold more cytosolic protein than the microsomal fraction, liver cytosolic protein was used five times as much as liver microsomal protein in the present study. When the combination of liver microsomes and cytosol was used as an enzyme source in the presence of the NADPH-generating system, the rate of 5-FU formation was low (Fig. 1C). However, by the addition of 5-chloro-2,4-dihydroxypyridine (100 μM), a DPD inhibitor (20), 5-FU formed by liver microsomes and cytosol was increased (Fig. 1D). When liver S9 (3.0 mg/ml) was used in the presence of 5-chloro-2,4-dihydroxytegafur (Fig. 1E), 5-FU formation from tegafur was almost similar to that by the combination of liver microsomes and cytosol (Fig. 1B) was used as an enzyme source in the presence of the NADPH-generating system and 5-chloro-2,4-dihydroxytegafur (100 μM). Results are presented as means of duplicate determinations.

Characterization of 5-FU Formation in Human Liver Cytosol. The rates of 5-FU formation from tegafur in the standard reaction mixtures containing human liver cytosol (sample HL-4) were increased linearly with cytosolic protein concentrations up to 2.5 mg/ml and durations up to 40 min. 5-FU formation was increased in a substrate concentration-dependent manner. Unless specified, an incubation time of 30 min, 2.5 mg/ml of cytosolic protein, and 1 mM tegafur were used to ensure the initial rate conditions for the formation of 5-FU. The apparent kinetic values (± SE) for 5-FU formation in human liver cytosol (HL-4) were calculated to be 16 ± 4 nmol/min/mg protein and 0.63 ± 0.14 nmol/min/mg protein, respectively, under the present conditions. On the other hand, the apparent kinetic values of microsomal 5-FU formation in HL-4 were 1.8 ± 0.3 nmol/min/mg protein and 0.46 ± 0.04 nmol/min/mg protein, respectively, indicating a 10-fold lower value than in the cytosol.

5-FU Formation Catalyzed by Human Liver Microsomes, Cytosol, and in Combination. To investigate the interindividual differences in microsomal and cytosolic 5-FU formation, 14 samples of human liver were examined at substrate concentrations of 100 μM and 1 mM. These concentrations were based on the reported clinical plasma concentrations in humans (21) and its 10-times higher concentration, respectively. The rates of 5-FU formation catalyzed by liver microsomes, cytosol, and in combination, in each sample are shown in Fig. 2, and the mean ± SD and the ranges for 14 samples are summarized in
Table 1 5-FU formation from tegafur catalyzed by human liver microsomes, cytosol, and in combination

Tegafur (100 μM or 1 mM) was incubated with human liver microsomes (0.5 mg/ml) in the presence of an NADPH-generating system, human liver cytosol (2.5 mg/ml) in the absence of the NADPH-generating system, and with the combination of human liver microsomes and cytosol in the presence of the NADPH-generating system and 5-chloro-2,4-dihydroxypyridine (100 μM), a DPD inhibitor. Results are presented as mean ± SD for 14 samples; values in parentheses indicate the ranges.

5-FU formation (pmol/min)

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<th>Condition</th>
<th>100 μM tegafur</th>
<th>1 mM tegafur</th>
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<tr>
<td>Microsomes</td>
<td>4.5 ± 2.4 (1.4–8.6)</td>
<td>36 ± 18 (15–72)</td>
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<tr>
<td>Cytosol</td>
<td>0.9 ± 0.3 (0.4–1.6)</td>
<td>18 ± 4 (11–24)</td>
</tr>
<tr>
<td>Microsomes and cytosol</td>
<td>5.5 ± 3.1 (2.2–11.1)</td>
<td>53 ± 20 (31–95)</td>
</tr>
<tr>
<td>Cytosol:microsomes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 ± 0.17 (0.10–0.64)</td>
<td>0.60 ± 0.32 (0.29–1.40)</td>
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<sup>a</sup> Cytosol:microsomes indicates the ratio of 5-FU formation activities by liver cytosol to those by liver microsomes in individual human samples.

Table 1. At 100 μM of tegafur, the mean rates of 5-FU formation by liver microsomes (4.5 pmol/min) were 5-fold higher than liver cytosol (0.9 pmol/min). The mean rates of 5-FU formation by the combination of liver microsomes and cytosol were 5.5 pmol/min. The individual ratios of cytosolic activities: microsomal activities were in the range of 0.10 to 0.64. On the other hand, the mean rates of 5-FU formation from 1 mM tegafur catalyzed by liver microsomes were twice as high as those by liver cytosol. The ratio of individual cytosolic activities: microsomal activities was in the range of 0.29–1.40. Particularly, the rates of liver microsomal and cytosolic 5-FU formation were similar in HL-4, and the liver cytosolic 5-FU formation activity in HG93 was higher than the liver microsomal activity (Fig. 2B). Interindividual differences among the 14 samples in 5-FU formation were 5-fold (at 100 μM tegafur) and 3-fold (1 mM tegafur) in the presence of liver microsomes and cytosol, respectively.

A Role of dThdPase in Human Liver Cytosolic 5-FU Formation. To investigate whether dThdPase and UrdPase in human liver cytosol catalyzed 5-FU formation, correlation and inhibition studies were carried out. Fig. 3 shows the relationships among 5-FU formation from tegafur and thymine from thymidine, uracil from uridine, and 5-FU formation from 5’t-dFUr'd in 14 samples of human liver cytosol. These activities were determined at a substrate concentration of 1 mM. 5-FU formation from tegafur was significantly correlated with thymine formation from thymidine catalyzed by liver cytosol (r, 0.93; P < 0.01; Fig. 3A). However, 5-FU formation from tegafur did not correlate with uracil formation from uridine, which has been reported to be mainly catalyzed by UrdPase (Ref. 13; r, 0.34; Fig. 3B). In addition, the rates of 5-FU formation from tegafur were significantly correlated with those from 5’t-dFUr’d (r, 0.68; P < 0.01; Fig. 3C).

The effects of dThdPase and UrdPase inhibitors on 5-FU formation from 1 mM of tegafur were examined (Table 2). Human liver cytosolic fractions of HL-1 and HL-10 were used, which showed low and high 5-FU formation activities, respectively, among the 14 samples tested. The rates of cytosolic 5-FU formation of either sample were inhibited moderately and strongly by the addition of 10 μM and 100 μM 5-chlorouracil, respectively. The K<sub>i</sub> values of 5-chlorouracil have been reported to be 38 μM and 11 μM for dThdPase and UrdPase, respectively (22). 6-Benzyl-2-thiouracil, a dThdPase-selective inhibitor (22), showed weak inhibition under the condition examined. The addition of TPI (0.1 μM), a strong inhibitor for dThdPase but not for UrdPase (17), efficiently inhibited cytosolic 5-FU formation. In contrast, 2,6-dihydroxypyridine (5 μM), a UrdPase-selective inhibitor (22), did not inhibit the rates of 5-FU formation in the human liver cytosol. By the addition of 500 μM of 2,6-dihydroxypyridine, cytosolic 5-FU formation was efficiently inhibited in both of the cytosol samples.

The formation of 5-FU from tegafur and that of thymine from thymidine catalyzed by E. coli dThdPase were 8 nmol/min/mg protein and 12,000 nmol/min/mg protein, respectively. These catalytic activities by recombinant human dThdPase were 80 nmol/min/mg protein for 5-FU formation and 10,000 nmol/min/mg protein for thymine formation, respectively. Taking
we used 5-chloro-2,4-dihydroxypyridine at the concentration of A; Table 1). Although the liver human liver samples (Fig. 2 (21), were 5-fold higher than those in the liver cytosol of 14 based on the reported clinical plasma concentrations in humans ml liver microsomes at a substrate concentration of 100 sufficiently.

DISCUSSION

These results into consideration, tegafur was biotransformed into 5-FU by dThdPase in human liver cytosol.

Tegafur has been clinically used for the treatment of various cancers. Recently, it is used with some modulators, for example, 5-chloro-2,4-dihydroxypyridine and potassium otastat (23) or uracil (24). Many enzymes are involved in the cytostatic effect of tegafur, a prodrug of 5-FU, although P450 and DPD are rate-limiting enzymes in activating to and inactivating of 5-FU (3, 7), respectively. In cancer chemotherapy, it would be useful to predict the efficacy of the drug in the patient before clinical trials. Therefore, studies concerning the prodrug activating enzymes would yield important information with regard to the possible clinical efficacy.

Two pathways are thought to exist in tegafur bioactivation, mainly in the microsomal fraction and partly in the cytosolic fraction (4–6, 8). In this study, we investigated the involvement of human liver cytosol in the biotransformation of tegafur into the active drug 5-FU to examine interindividual differences in 5-FU formation. When 5-FU formation by the combination of liver microsomes and cytosol was examined, 5-chloro-2,4-dihydroxypyridine was added. Because 5-chloro-2,4-dihydroxypyridine has been reported to be a strong DPD inhibitor with a Ki value of 0.36 μM for 5-FU degradation by rat liver extract (20), we used 5-chloro-2,4-dihydroxypyridine at the concentration of 100 μM, which was thought to inhibit human DPD activity sufficiently.

In the present study, the mean 5-FU formation activities in liver microsomes at a substrate concentration of 100 μM tegafur, based on the reported clinical plasma concentrations in humans (21), were 5-fold higher than those in the liver cytosol of 14 human liver samples (Fig. 2A; Table 1). Although the liver cytosolic 5-FU formation was similar to, or higher than, the liver microsomal 5-FU formation in some of the human liver samples at 1 mM tegafur, the mean rates of 5-FU formation in liver microsomes were 2-fold higher than those in liver cytosol (Fig. 2B; Table 1). From these results, it is suggested that 5-FU formation from tegafur in human liver was mainly catalyzed by microsomes at low concentrations of tegafur, and the contribution of cytosol would become larger at higher concentrations. At 100 μM tegafur, the ratios of the maximum:minimum 5-FU formation were 6 and 4 in human liver microsomes and cytosol, respectively. The interindividual differences of 5-FU formation in the combination of liver microsomes and cytosol were still observed (5-fold). The 5-FU formation by liver S9 was comparable with that of the combination of liver microsomes and cytosol, as indicated in Fig. 1, D and E. There were the similar individual differences in 5-FU formation catalyzed by the seven human liver S9 samples available in this study (data not shown).

It is known that P450 in human liver microsomes catalyze 5-FU formation from tegafur (6, 7), and recently, we clarified the roles of CYP1A2, CYP2A6, and CYP2C8 in liver microsomal 5-FU formation (8). There are large interindividual variations in the contents and activities of several P450 forms (25). Genetic polymorphisms are reported in CYP1A2 (26, 27) and CYP2A6 (28, 29). An enhanced expression of P450 has been shown in human cancer tissues (30, 31). In contrast, a reduction in P450-mediated drug metabolism in cancer patients has been recently reported (32). Therapeutically used IFN and interleukin-2 have been shown to decrease the liver microsomal P450 in humans (33, 34). These contradictory findings suggest that the levels of P450 in tumor tissues may be a key factor for predicting the efficacy of tegafur.

It has been reported that dThdPase catalyzed 5-FU formation from tegafur (10). In the present study, we clarified the role of dThdPase (not UrdPase) in cytosol by the correlation between liver cytosolic 5-FU formation from tegafur and typical dThdPase activities (Fig. 3) and by the effects of dThdPase and UrdPase inhibitors on liver cytosolic 5-FU formation (Table 2). It has been reported that the levels or activities of dThdPase in human breast and lung cancer tissues are 10–20 times higher than in normal tissues (9, 12). Apparently higher levels of dThdPase in various tumor tissues including stomach, colon, and ovary, compared with normal tissues, have also been shown (11). The existence of a genetic polymorphism of dThdPase is

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* HL, human liver (sample).

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not known, but it has been reported that there are large interindividual variations of dThdPase levels (10- to 320-fold) in various cancer tissues including liver (35). The levels of dThdPase have been shown to be up-regulated by some factors like tumor necrosis factor α and anticancer drugs including paclitaxel and cyclophosphamide (36, 37). In addition, differences in substrate specificity have been observed between human liver dThdPase and human placenta dThdPase, which suggests the presence of tissue-specific isozymes (13). Thus, the levels and/or activities of dThdPase in cytosol fractions would be variable in various tissues or disease conditions.

From the present results and our recent findings (8), using human liver samples and recombinant enzymes, different contributions of CYP1A2, CYP2A6, and CYP2C8, as well as dThdPase, were suggested in the bioactivation of tegafur into 5-FU in individual human livers. The participation of many kinds of enzymes in tegafur activation suggested the presence of compensatory bioactivation, even when one of these enzymes is more scarce or deleted in some individuals. However, certain interindividual differences in 5-FU formation were clearly observed in the presence of human liver microsomes and cytosol. There would be interindividual variations in the levels of each of the microsomal P450 and cytosolic dThdPase involved in 5-FU formation from tegafur in human liver. These results suggest that the determination of the activities or levels of multiple enzymes in patients would be important for improving clinical cancer therapy with tegafur.

In conclusion, we demonstrated that 5-FU formation from tegafur in human liver was mainly catalyzed by microsomes, but the contribution of cytosolic 5-FU formation would be important in some individuals. We examined the involvement of microsomal P450 and cytosolic dThdPase in tegafur activation into 5-FU with normal human liver samples in this study. Further investigation on the involvement of microsomal and cytosolic 5-FU formation in tumor cells will be necessary. These findings may be helpful for the prediction of the pharmacokinetics or susceptibility of tegafur in vivo.

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Involvement of Microsomal Cytochrome P450 and Cytosolic Thymidine Phosphorylase in 5-Fluorouracil Formation from Tegafur in Human Liver

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