Tegafur, an anticancer prodrug, is reported to be bioactivated to 5-fluorouracil (5-FU) by cytochrome P450 (P450) enzymes. Liver microsomal P450 enzymes involved in the biotransformation of tegafur into 5-FU in rats and the effect of tegafur in vivo on P450 levels in rats were investigated. Of 12 cDNA-expressed rat P450 enzymes tested, CYP1A2, CYP3A1, and CYP2C11 had high 5-FU formation rates from 100 μM and 1.0 mM tegafur concentrations. The contributions of CYP1A, CYP2C, and CYP3A enzymes to 5-FU formation in male rat liver microsomes were supported by immunoinhibition studies. 5-FU formation from tegafur, at substrate concentrations of 100 μM and 1.0 mM, was increased by intraperitoneal treatment of tegafur (50 mg/kg for 5 days) as well as by β-naphthoflavone, phenobarbital, and dexamethasone. Orally administered tegafur (100 mg/kg daily for 20 days) caused the induction of CYP2B (5-fold) and of CYP1A and CYP3A (≈2-fold) in rat liver microsomes. These results suggest that CYP1A and CYP3A enzymes, autoinduced by tegafur, have important roles in 5-FU formation from tegafur in rat liver microsomes. Coadministration of tegafur and P450-inducing drugs could markedly enhance the biotransformation of tegafur into 5-FU via P450 induction.

Materials and Methods

Chemicals. Tegafur (>99.9%), 5-FU, and other chemicals used in this study were obtained from sources described previously and were of the highest quality commercially available (Komatsu et al., 2000a,b).

Enzyme Preparations. Male and female Wistar rats were obtained from Japan SLC (Hamamatsu, Japan). Male rats (7 weeks old) were treated with tegafur (100 mg/kg) suspended in 0.5% carboxymethyl cellulose sodium salt solution via oral administration for 20 days. Rats were starved for 15 h before decapitation on the 21st day (10 weeks old). Liver microsomes were prepared as described previously (Komatsu et al., 2000a). In separate experiments, male rats (6 weeks old) were intraperitoneally treated daily for 5 days with tegafur (50 mg/kg) as well as typical P450 inducers including β-naphthoflavone (50 mg/kg, CYP1A), phenobarbital (80 mg/kg, CYP2B and CYP3A), isoniazid (100 mg/kg, CYP2E1), and dexamethasone (50 mg/kg, CYP3A). Liver microsomes from these rats and untreated male and female rats at 7 weeks old were prepared as mentioned above. Recombinant P450 enzymes expressed in microsomes of insect cells infected with baculovirus containing rat P450 and NPR cDNA inserts (Supersomes) were obtained from GENTEST (Woburn, MA). We used them without further modifications in this study. Catalytic activities by those P450 enzymes are provided in the data sheets by the manufacturer. Anti-rat CYP1A2, anti-rat CYP2C13, and anti-CYP3A2 antibodies for inhibition studies were obtained from Daiichi Pure Chemicals (Tokyo, Japan). No reactivities of these antibodies to the other subfamily members of rat P450 enzymes are provided in the data sheets by the manufacturer.

Enzyme Assays. 5-FU formation activities from tegafur were determined according to the high-performance liquid chromatography methods described previously (Komatsu et al., 2000b). Since a trace peak corresponding to 5-FU...
concentrations of tegafur after treatment in humans (~0.01% of the substrate) was observed in the absence of the NADPH-generating system, the background levels were subtracted for calculating 5-FU formation catalyzed by P450 enzymes at each substrate concentration. Other oxidative metabolites (trans-4′-hydroxy-tegafur, cis-4′-hydroxy-tegafur, trans-3′-hydroxy-tegafur, cis-3′-hydroxy-tegafur, and 4′,5′-dehydro-tegafur) were not produced by recombinant rat P450 enzymes and rat liver microsomes in the present assay conditions. O-Deethylation of 7-ethoxycoumarin (CYP1A and CYP2B) and ethoxyresorufin (CYP1A), aniline p-hydroxylation (CYP2E1), and testosterone 6β-, 16α-, and 16β-hydroxylation (CYP3A, CYP2C, and CYP2B, respectively) were determined as described previously (Komatsu et al., 2000). The concentrations of total P450 and cytochrome b5 (Omura and Sato, 1964), individual P450 enzymes (Shimada et al., 1994), NPR (Parikh et al., 1997), and protein (Lowry et al., 1951) were determined as described previously.

Statistical Analysis. The kinetic analysis of 5-FU formation was estimated using a computer program (KaleidaGraph program from Synergy Software, Reading, PA) designed for nonlinear regression analysis. Statistical analysis was carried out using the computer program Instat (GraphPad Software, San Diego, CA) designed for Student’s t test after the assumption of equal variance with an F test.

Results and Discussion

In our preliminary experiments with recombinant rat CYP1A2 and CYP3A1, the activities of 5-FU formation at 1.0 mM tegafur increased linearly for up to 40 min of incubation time and up to 40 pmol of P450/ml. Based on the present results and reported plasma concentrations of tegafur after treatment in humans (~100 μM) (Kanamitsu et al., 2000), a substrate concentration of 100 μM tegafur and a 10-fold higher concentration, 1.0 mM, were mainly used in this study. Unless specified, an incubation time of 30 min and a P450 concentration of 20 pmol/ml were used to ensure the initial rate conditions for the formation of 5-FU. Twelve recombinant rat P450 enzymes were used to determine which P450 enzymes were active in catalyzing the 5-FU formation (Table 1). CYP1A2, CYP3A1, and CYP2C11 showed high activities of 5-FU formation at both substrate concentrations. CYP2C6 and CYP3A2 also showed weak activities. However, CYP1A1, CYP2A2, CYP2B1, CYP2C12, CYP2C13, CYP3D1, and CYP2D2 showed very low or undetectable activities. 5-FU formation catalyzed by CYP1A2, CYP2C11, and CYP3A1 was almost linearly increased in a substrate concentration-dependent manner (0.1–2.0 mM).

The rates of 5-FU formation from tegafur (1 mM) in standard reaction mixtures containing liver microsomes from untreated male rats were increased linearly up to 40 min with microsomal protein concentrations up to 1.0 mg/ml. The 5-FU formation at 1.0 mM tegafur by liver microsomes from untreated male rats was inhibited by SKF-525A or metyrapone (50 μM) by ~80% and by anti-CYP1A1, anti-CYP2C2, or anti-CYP3A antibodies (10 mg of IgG/nmol of P450) by ~30%. 5-FU formation at 100 μM and 1.0 mM tegafur by male rat liver microsomes (7 weeks old) was greater than for female rat liver microsomes (Fig. 1A). Among typical P450 inducers administered intraperitoneally, dexamethasone caused the most induction of 5-FU formation, followed by phenobarbital and β-naphthoflavone. Autoinduction by tegafur treatment (50 mg/kg for 5 days) was also observed when 1.0 mM tegafur was used as a substrate (Fig. 1A). 5-FU formation activities were increased in a substrate concentration-dependent manner in liver microsomes from untreated and dexamethasone-treated rats (7 weeks old). The apparent Km (μM ± S.E.) and Vmax (nmol/min/mg of protein ± S.E.) values for 5-FU formation in liver microsomes from untreated and dexamethasone treated rats were calculated to be 2.7 ± 0.7 and 0.50 ± 0.21, and 2.3 ± 0.4 and 2.5 ± 0.3, respectively. These results suggested that CYP1A1 and CYP3A enzymes had important roles for 5-FU formation in rat liver microsomes.

To examine whether tegafur induced tegafur-bioactivation in detail, male rats were orally treated with tegafur (100 mg/kg for 20 days). Chronic oral administration of tegafur to rats induced the 5-FU formation by 1.7- and 2.2-fold at concentrations of 100 μM and 1.0 mM tegafur, respectively (Fig. 1B). 5-FU formation activities were increased in a substrate concentration-dependent manner in liver microsomes from untreated and tegafur-treated rats (10 weeks old). The Km (μM ± S.E.) and Vmax (nmol/min/mg of protein ± S.E.) values for liver microsomes from

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**TABLE 1**

5-FU formation from tegafur catalyzed by recombinant rat P450 enzymes

![Graph A](Image 94x247 to 233x319)

**Fig. 1. 5-FU formation from tegafur catalyzed by rat liver microsomes.**

Tegafur (100 μM and 1.0 mM) was incubated at 37°C for 30 min with rat liver microsomes (0.5 mg of protein/ml) in the presence of a NADPH-generating system. Apparent control activities (0.01 and 0.32 nmol/min/nmol of P450 in 100 μM and 1.0 mM of tegafur) in the presence of insect microsomes expressing only NPR and cytochrome b5 were subtracted. Results are presented as means of duplicate determinations.
and coumarin 7-hydroxylation (Yamazaki et al., 1994). CYP3A was active to varying extents in both species. CYP1A2 was also active.

With regard to modulating P450 functions, 5-FU (a single intraperitoneal dose of 120 mg/kg) has been reported to suppress the levels of CYP2C11 and CYP3A enzymes and/or activities of steroid 6β-, 16α-, and 2α-hydroxylation to approximately 40 to 50% in rat liver microsomes (Afsar et al., 1996). On the other hand, a single intraperitoneal dose of 5-FU (120 or 200 mg/kg) did not affect the total P450 contents or testosterone 6β- and 2β-hydroxylation activities (Stupans et al., 1995; McLeod et al., 1998). However, there is no information with regard to the induction or suppression of individual P450 enzymes by tegafur. Here we show that orally administered tegafur efficiently induced the CYP2B enzymes, followed by CYP3A and CYP1A enzymes as determined immunochromically. Total P450 and NPR contents were also increased in rat liver microsomes (Fig. 2).

Drug oxidation activities catalyzed by CYP3A, CYP2B, and CYP1A were also increased, supporting the other evidence for induction. This is the first report of the induction of P450 contents, especially CYP2B, CYP3A, and CYP1A enzymes by tegafur, although the mechanism of induction of three different P450 enzymes is not clear.

In conclusion, we showed that tegafur is able to induce CYP1A, CYP2B, and CYP3A enzymes in rat liver microsomes and that CYP1A and CYP3A enzymes induced by tegafur have important roles in liver microsomal 5-FU formation from tegafur in rats. In cancer chemotherapy, several anticancer drugs may be coadministered. Tegafur has been occasionally administered with methotrexate and cyclophosphamide or with doxorubicin methotrexate, and tannoxifen for the treatment of breast or renal cancer (Wada et al., 1993; Ribas et al., 1998). Chronic administration of tegafur would affect the pharmacokinetics of tegafur itself and other coadministered drugs, including antecancer agents via P450 induction. These findings may be useful for the basic understanding of tegafur-induced drug interactions.

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References


