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Depression of phenytoin metabolic capacity by 5-fluorouracil and doxifluridine in rats

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Abstract

It has been found in clinical practice that the serum level of phenytoin, of which metabolism is mediated by hepatic CYP2C enzymes, was markedly elevated by co-administration of 5-fluorouracil (5-FU) and doxifluridine (5′-deoxy-5-fluorouridine; 5′-DFUR), a prodrug of 5-FU, but the detailed mechanisms are unclear. A study using rats was undertaken to examine the effects of 5-FU and 5′-DFUR on phenytoin metabolism in hepatic microsomes and phenytoin pharmacokinetics in-vivo. Neither 5-FU nor 5′-DFUR exhibited direct inhibitory effects on hepatic microsomal phenytoin *p*-hydroxylation, a major metabolic route catalysed by CYP2C in rats, as in humans. 5-FU and 5′-DFUR were injected intraperitoneally into male rats as single doses (1.68 mmol kg^{-1}) and repeated doses (0.24 mmol kg^{-1} for 7 days). Control rats received vehicle alone. A significant reduction in the activity of phenytoin *p*-hydroxylation was observed 4 days after the last administration irrespective of the agents and their treatment regimens, although the activity was unchanged on Day 1. Pharmacokinetic analysis of phenytoin revealed that the elimination rate constant and the total clearance was decreased by 70–75% in both the 5′-DFUR-treated and 5-FU-treated rats, indicating that the decrease in the metabolic capacity of phenytoin was responsible for the change in phenytoin disposition in-vivo. On the other hand, 5-FU significantly depressed the total P450 content, NADPH cytochrome c reductase activity and activities of progesterone hydroxylations. However, the depressive effects of 5′-DFUR were not very potent relative to those of 5-FU, which can be explained by the fact that 5-FU is derived from 5′-DFUR to only a small extent. According to a recent report, phenytoin *p*-hydroxylation and progesterone 2α-/21-hydroxylations share common CYP2C enzymes as their catalysts. Because there was a difference in the modulation profiles between phenytoin *p*-hydroxylation and progesterone 2α-/21-hydroxylations after exposure to 5′-DFUR, 5′-DFUR might modulate phenytoin metabolism without loss of catalytic ability for other substrates, unlike 5-FU. The present study suggested that the down-regulation of hepatic CYP2C enzymes occurs by 5-FU exposure even at a low level, and provided a fundamental explanation for the drug interaction encountered in clinical practice.

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Introduction

5-Fluorouracil (5-FU) is a pyrimidine analogue that is widely used to treat a variety of solid tumours such as breast, gastric, colorectal, head and neck, and pancreatic cancers. Doxifluridine (5′-deoxy-5-fluorouridine; 5′-DFUR) is an oral active prodrug of 5-FU, and exerts its antitumour activity after being converted into 5-FU by pyrimidine nucleoside phosphorylase, which is expressed at a higher level in tumour tissues than in the adjacent normal tissue (Armstrong & Diasio 1980; Ishitsuka et al 1980; Kono et al 1983). As the therapeutic index of 5′-DFUR is expected to be higher than that of 5-FU, owing to the preferential generation of 5-FU in the tumour tissues (Uehara et al 1985), 5′-DFUR is frequently used in the treatment of malignancies responsive to 5-FU (Tsukagoshi 1987; Calabresi & Repetto 1991; Tsuruta et al 2001). Despite the efficacy of these agents, a significant drug interaction of 5-FU with phenytoin was observed in clinical practice (Harada et al 1990; Gilbar & Brodribb 2001) and, recently, we also

reported a case showing neurological symptoms caused by over 4-fold elevation of the serum phenytoin concentration about 1 month after commencing co-administration with 5′-DFUR (Konishi et al 2002). Since phenytoin is eliminated principally by metabolic conversion via *p*-hydroxylation in the liver, with less than 5% of an administered dose being excreted in urine in an unchanged form (Levy 1995), the pharmacokinetic behaviour of phenytoin is highly dependent on the change in the metabolic capacity of the *p*-hydroxylation process (Bajpai et al 1996). Therefore, the interactions between phenytoin and 5-FU/5′-DFUR can be interpreted as the result of inhibition of phenytoin metabolism in the liver; however, the detailed mechanism remains unclear.

The present study aimed at understanding the inhibitory effects of 5-FU and 5′-DFUR on the activities of phenytoin *p*-hydroxylation and several related enzymes in hepatic microsomes and on the phenytoin disposition using rats. In rats, as in humans, the major metabolic pathway of phenytoin is hepatic *p*-hydroxylation, which accounts for most total body clearance (CL_{tot}) (Umeda & Inaba 1978).

Materials and Methods

Chemicals and animals

5′-DFUR was kindly donated by Nippon Roche K. K. (Tokyo, Japan). 5-FU was purchased from Sigma Chemical Co. (St Louis, USA), and sodium salt of phenytoin was from Attest Inc. (Kyoto, Japan). Other chemicals and solvents were of analytical grade. Male Sprague–Dawley rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). The rats were acclimated for at least 2 days after their arrival, and were assigned to experimental groups at 6–8 weeks of age (200–250 g). They were housed in a clean room under standard conditions (temperature 23 ± 2°C, relative humidity 55 ± 10%, 12-h light–dark cycle) with free access to drinking fluid and rodent chow (except for fasting for 24 h before killing). The rats used in this study were handled in accordance with the Guidelines for Animal Experimentation of Shiga University of Medical Science, and the experimental protocol was approved by the Animal Care and Use Committee of the institution.

Animal treatment

5-FU and 5′-DFUR were dissolved in saline, and were injected intraperitoneally at doses of 0.24 mmol kg^{-1} for 7 consecutive days, or 1.68 mmol kg^{-1} once. Control rats were given vehicle alone at the same time.

Preparation of rat hepatic microsomes

The rats were killed by cervical dislocation under ether anaesthesia, 1, 4 and 7 days after the last injection. Livers from untreated and 5-FU- and 5′-DFUR-treated rats were perfused with ice-cold 1.15% potassium chloride and then homogenized in the same salt solution (1:4, w/v). The homogenate was centrifuged at 10000 *g* for 15 min, and the supernatant fractions were centrifuged at 105000 *g* for 60 min to obtain microsomes. The pellet was suspended in 0.1 M sodium/potassium phosphate buffer, pH 7.4, to a final concentration of 1–5 mg protein mL^{-1}. The protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

Biochemical analysis

The enzyme reaction was performed in 1 mL of a mixture of an NADPH-generating system consisting of 0.5 mM $NADP^+$, 5 mM glucose-6-phosphate, 5 mM $MgCl_2$ and 2 units of glucose-6-phosphate dehydrogenase, 67.5 mM sodium/potassium phosphate buffer (pH 7.4), microsomes (1 mg mL^{-1}) and substrate (50 μM phenytoin or 100 μM progesterone). The reaction was started by adding the microsomal suspension, and the incubation was conducted at 37°C with constant shaking for 10 min. The phenytoin hydroxylation reaction was stopped by the addition of 4 mL ethyl acetate, and 250 pmol phenobarbital was added as an internal standard. The reaction of progesterone oxidation (2α-hydroxylation as a probe for CYP2C6, 6β-hydroxylation for CYP3A2, 21-hydroxylation for CYP2C11 and androstenedione formation mediated partially by the CYP2C subfamily) was stopped by adding 4 mL dichloromethane, and 400 pmol cortisone acetate was added as an internal standard. The extracts were evaporated to dryness at 37°C under reduced pressure, the residue was reconstituted in 200 μL methanol and a 20-μL sample was subjected to high-performance liquid chromatography (HPLC). It was confirmed that the enzyme reactions showed linearity with respect to protein concentration and incubation time, at least up to 1 mg mL^{-1} and 10 min, respectively.

The P450 content in hepatic microsomes was measured as described by Omura & Sato (1964). The NADPH-cytochrome c reductase activity was determined by the method of Peters & Fouts (1970).

HPLC conditions

The chromatographic assembly (Shimadzu, Kyoto, Japan) consisted of an LC-10A pump, an SPD-10A UV spectrophotometric detector and a C-R6A Chromatopac integrator. Separation of the metabolites, substrate and internal standard was performed on a Shim-pak CLC-ODS column (particle size 5 μm, 150 × 4.6 mm inner diameter; Shimadzu) at ambient temperature. 5-(4-Hydroxyphenyl)-5-phenylhydan toin (4′-HPPH), the *p*-hydroxylated metabolite of phenytoin, was measured according to the procedure of Schellens et al (1990), using 50% methanol (v/v,%) as the mobile phase. The flow rate was set at 0.5 mL min^{-1}, and the column effluent was monitored at 214 nm. On the other hand, 2α-, 6β- and 21-hydroxyprogesterone and androstenedione were determined by the gradient HPLC methods of Arlotto et al (1991) and Cribb et al (1995) with modification. The mobile phases consisted of solvent A (methanol/acetonitrile/water, 26:26:48, v/v) and solvent B (100% methanol). Isocratic elution with solvent A was first performed for 15 min after sample injection, and the proportion of solvent B was increased

linearly from 0% to 100% over 10 min, then held at 100% for an additional 5 min. The flow rate was 1 mL min^{-1}, and the analytes were detected at 254 nm. The metabolites were identified by comparison with retention time of the authentic standards, and were quantified using the peak area ratio to the internal standard.

Pharmacokinetic study of phenytoin after oral administration to rats

Phenytoin sodium was dissolved in saline, and rats fasted overnight received an oral dose of phenytoin (250 mg kg^{-1} as free base) over a period of approximately 10 s by use of an oral feeding tube 4 days after single intraperitoneal treatment with 5-FU (1.68 mmol kg^{-1}), 5′-DFUR (1.68 mmol kg^{-1}) and vehicle alone. Blood samples were drawn without restriction from a neck vein under slight ether anaesthesia at 1, 2, 4, 6, 8 and 12 h after administration, and the serum fraction was separated by centrifugation. The rats were unconstrained after phenytoin administration. Phenytoin concentration in serum was determined by a fluorescence polarization immunoassay technique using a TDxFLx system kit (Dainabot Laboratories, Tokyo, Japan) according to the directions provided by the manufacturer. Non-compartment pharmacokinetic parameters of phenytoin were obtained by moment analysis as described below. The elimination rate constant at the terminal phase (Ke) was determined by linear regression of the log-linear portion of plots of serum concentration against time. Elimination half-life ($t_{\frac{1}{2}}$) was calculated as being equal to 0.693 Ke^{-1}. CL_{tot} was determined by dividing oral dose (250 mg kg^{-1}) by the area under the plots of serum concentration against time after oral phenytoin administration (AUC). AUC was calculated by the linear trapezoidal integration up to the last measured concentration, and was extrapolated to infinity by addition of the ratio of the last measured concentration to the calculated Ke. The apparent volume of distribution at steady state (Vd_{ss}) was determined by multiplying CL_{tot} by the mean residence time after phenytoin administration.

Statistical analysis

The results are expressed as mean ± s.d. The data were subject to a one-way analysis of variance followed by multiple comparisons using the Tukey's test. A value of $P < 0.05$ was considered significant.

Results

To determine whether 5-FU and 5′-DFUR have direct inhibitory effects on phenytoin *p*-hydroxylase activity, the enzyme reaction was carried out in the presence of these agents using untreated rat hepatic microsomes. No inhibition was observed at concentrations up to at least 1 mM of both agents in-vitro (data not shown). Subsequently, we examined changes in the phenytoin *p*-hydroxylase activity in hepatic microsomes after repeated and single doses of 5-FU and 5′-DFUR to rats. With respect to 5-FU administration, the two methods (single and repeated administrations) were selected to mimic the clinical treatment regimen. In the case of the repeated dosing, 5-FU was given daily at 30 mg kg^{-1} (0.24 mmol kg^{-1}) for 7 days, as this dosage was reported to exert maximal anticancer activity without organ toxicity (Miyazaki et al 1974). Based on this evidence, the dose of 5′-DFUR was decided as 0.24 mmol kg^{-1} so as to maintain molar equivalence to 5-FU. In the case of single administration, rats received 1.68 mmol kg^{-1} of the two agents, which was equal to the total dose used in the experiment of repeated treatment. Figure 1 shows the hepatic microsomal phenytoin *p*-hydroxylase activity 1, 4 and 7 days after cessation of the single and repeated treatments with the two agents. There was no change in the activity 1 day after the last injection, regardless of the treatment regimen. When administered repeatedly, 5-FU and 5′-DFUR led to 30% and 39% decreases in the enzyme activity, respectively, on Day 4 after the last injection. Seven days after the last injection, the activities of the 5′-DFUR-treated rats showed a tendency toward returning to the basal level, although the depression of the activities was sustained in the 5-FU-treated rats. Regarding the single treatment regimen, the activities were decreased by 42% and 48% 4 days after the administration of 5-FU and 5′-DFUR, respectively. The activity tended to recover 7 days after the administration.

Pharmacokinetic analysis of phenytoin was performed to examine whether the alteration in the phenytoin metabolism in hepatic microsomes was closely related to the phenytoin disposition in-vivo. The serum phenytoin concentration–time profiles in rats treated with single doses of 5-FU, 5′-DFUR or vehicle alone are shown in Figure 2. The two agents markedly increased the serum levels of phenytoin. Table 1 shows the pharmacokinetic parameters of phenytoin. Treatment with 5-FU and 5′-DFUR significantly reduced the Ke by 70% and 73%, and similarly decreased the CL_{tot} by 73% and 75%, respectively, but no significant change was found in the Vd_{ss}. There was no significant difference in the pharmacokinetic parameters between 5-FU-treated and 5′-DFUR-treated rats.

Table 2 shows the changes in bodyweight, liver weight, hepatic P450 content and NADPH cytochrome c reductase activity after treatment of rats with 5-FU and 5′-DFUR. 5-FU produced a significant decrease in hepatic total P450 content measured by CO-difference spectra on Days 4 and 7, whereas the depressive effect of 5′-DFUR on P450 expression was not strong compared with that of 5-FU, with only a slight decrease being observed on Day 4 after administration. The NADPH cytochrome c reductase activity of the 5-FU-treated rats was decreased by 28% on Day 4 and 30% on Day 7 after the last administration of the repeated treatment, and was decreased by 28% on Day 4 after the single administration. In contrast, no significant change in the NADPH cytochrome c reductase activity was observed in the 5′-DFUR-treated rats throughout the experimental period. On the other hand, neither 5-FU nor 5′-DFUR affected the liver weight, and no significant difference in bodyweight was observed among the treatment groups, except that there was a slight decrease in the rats receiving the repeated dose of 5-FU.

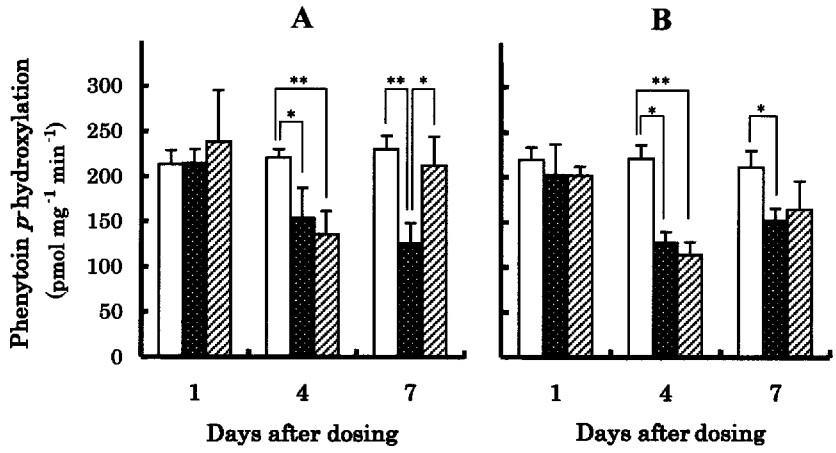

Figure 1 Effects of 5-FU and 5′-DFUR treatments on phenytoin *p*-hydroxylation in rat hepatic microsomes. Rats received repeated doses (0.24 mmol kg^{-1} day^{-1} for 7 days) (A) or single doses (1.68 mmol kg^{-1}) (B) of 5-fluorouracil (dotted columns) and 5′-deoxy-5-fluorouridine (hatched columns), or saline alone (open columns). Hepatic microsomes were prepared 1, 4 and 7 days after the last dose. Enzyme reaction was carried out at a substrate concentration of 50 μM. Data are given as mean±s.d. of four experiments. *$P < 0.05$; **$P < 0.01$.

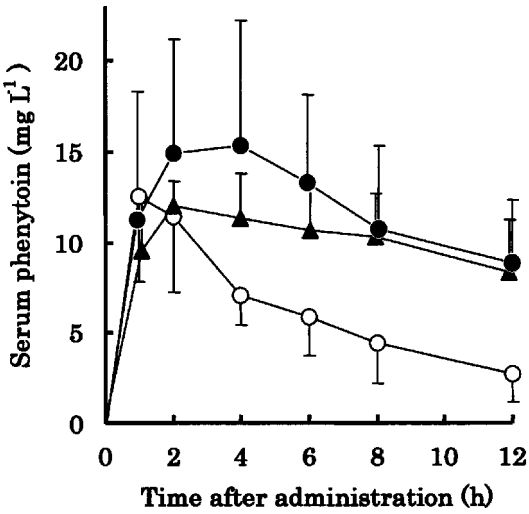

Figure 2 Serum concentration–time profile of phenytoin in rats treated with 5-FU and 5′-DFUR. Rats received oral phenytoin (250 mg kg^{-1}) 4 days after treatment with 1.68 mmol kg^{-1} 5-fluorouracil (●), 5′-deoxy-5-fluorouridine (▲), or saline (○). Data are given as mean±s.d. of four experiments.

Table 3 shows aspects of alteration in the activities of progesterone oxidations in rats after repeated dosing of 5-FU and 5′-DFUR. Progesterone 2α-hydroxylation was markedly depressed beginning on Day 1 after the last administration of 5-FU, whereas 5′-DFUR did not produce a significant change in its enzyme activity. The activities of progesterone 6β-hydroxylation, progesterone 21-hydroxylation and androstenedione formation were also significantly decreased by treatment with 5-FU, but there was no significant reduction in progesterone oxidation activities observed in 5′-DFUR-treated rats, except that progesterone 6β-hydroxylation was decreased on Day 4.

Discussion

Phenytoin is a commonly used anti-epileptic agent with a narrow therapeutic window. In humans, phenytoin is almost completely metabolized to 4′-HPPH, the *p*-hydroxylated form of phenytoin, primarily by CYP2C9, and to a minor extent by CYP2C19 in the liver, and this metabolic conversion accounts for most of the total pheny-

Table 1 Pharmacokinetic parameters of phenytoin in 5-fluorouracil (5-FU)- and 5′-deoxy-5-fluorouridine (5′-DFUR)-treated rats.

Parameter	Treatment		
	Control (saline)	5-FU (1.68 mmol kg^{-1})	5′-DFUR (1.68 mmol kg^{-1})
Ke (h^{-1})	0.135±0.026	0.041±0.012**	0.037±0.017**
$T_{\frac{1}{2}}$ (h)	5.3±1.1	18.0±5.9**	21.6±7.8**
Vd_{ss} (L kg^{-1})	21.2±5.5	19.9±10.7	19.7±3.5
AUC (mg h L^{-1})	96±34	371±135*	402±141*
CL_{tot} (L h^{-1} kg^{-1})	2.86±0.93	0.78±0.39*	0.72±0.38*

Results are mean±s.d. of four experiments. *$P < 0.05$ vs control; **$P < 0.01$ vs control.

Table 2 Effects of treatment with 5-fluorouracil (5-FU) and 5′-deoxy-5-fluorouridine (5′-DFUR) on bodyweight, liver weight, hepatic microsomal total P450 content and NADPH cytochrome c reductase activity.

Day after dosing	Treatment	Bodyweight (g)	Liver weight (g)	P450 content (nmol mg^{-1})	NADPH cytochrome c reductase activity (nmol mg^{-1} min^{-1})
Repeated administration					
1	Control	285±30	19.3±1.7	0.95±0.07	263±46
	5-FU	195±32†	15.4±4.7	0.93±0.11	225±15
	5′-DFUR	258±11	18.0±1.4	0.94±0.11	293±30
4	Control	288±21	18.4±1.4	0.91±0.05	263±15
	5-FU	225±10‡	16.4±1.2	0.62±0.14**	190±37‡
	5′-DFUR	279±10	18.6±0.7	0.77±0.06	271±14
7	Control	302±21	19.3±1.9	0.92±0.08	243±13
	5-FU	237±21†	18.4±1.5	0.60±0.11‡	170±19§
	5′-DFUR	293±17	20.1±2.3	0.94±0.09	250±51
Single administration					
1	Control	273±32	19.8±2.3	0.94±0.04	271±23
	5-FU	267±49	18.9±3.0	0.91±0.07	242±47
	5′-DFUR	266±52	18.3±2.5	1.02±0.06	267±22
4	Control	290±41	21.7±4.5	0.94±0.04	267±19
	5-FU	236±21	15.4±1.0	0.82±0.04*	192±38*
	5′-DFUR	275±27	20.4±1.7	0.83±0.05*	232±28
7	Control	275±21	19.5±1.6	0.96±0.02	272±36
	5-FU	240±36	17.4±3.1	0.66±0.08‡	246±33
	5′-DFUR	277±26	19.8±2.6	0.90±0.03	269±52

Rats received repeated doses (0.24 mmol kg^{-1} day^{-1}) of 5-FU and 5′-DFUR, or saline for 7 days (repeated administration), and single doses (1.68 mmol kg^{-1}) of 5-FU and 5′-DFUR, or saline (single administration). Hepatic microsomes were prepared 1, 4 and 7 days after the last dose. Results are mean±s.d. of four experiments. *$P < 0.05$ vs control; **$P < 0.01$ vs control; †$P < 0.01$ vs control and $P < 0.05$ vs 5′-DFUR treatment; ‡$P < 0.01$ vs control and 5′-DFUR treatment; §$P < 0.05$ vs 5′-DFUR treatment.

Table 3 Effects of 5-fluorouracil (5-FU) and 5′-deoxy-5-fluorouridine (5′-DFUR) treatments on metabolic conversion of progesterone in rat hepatic microsomes.

Day after dosing	Treatment	Enzyme activity (pmol mg^{-1} min^{-1})			
		Progesterone 2α-hydroxylation	Progesterone 6β-hydroxylation	Progesterone 21-hydroxylation	Androstenedione formation
1	Control	1100±260	697±175	80±11	189±35
	5-FU	440±140‡	707±67	90±15	167±30
	5′-DFUR	1250±270	756±125	84±11	223±55
4	Control	1040±240	645±62	81±9	175±22
	5-FU	359±62‡	359±100*	57±13	96±25*
	5′-DFUR	913±111	418±84*	73±15	152±50
7	Control	952±125	714±149	73±12	185±36
	5-FU	235±90‡	343±90†	41±12*	92±32*
	5′-DFUR	885±78	627±91	67±13	152±47

Rats received repeated doses (0.24 mmol kg^{-1} day^{-1} for 7 days) of 5-FU, 5′-DFUR, or saline alone. Hepatic microsomes were prepared 1, 4 and 7 days after the last dose. Enzyme reaction was carried out at a substrate concentration of 100 μM. Data are given as mean±s.d. of four experiments. *$P < 0.05$ vs control; †$P < 0.01$ vs control and $P < 0.05$ vs 5′-DFUR treatment; ‡$P < 0.01$ vs control and 5′-DFUR treatment.

toin elimination (Bajpai et al 1996). Accordingly, the CL_{tot} of phenytoin is susceptible to alteration by co-administration with other drugs that affect hepatic CYP2C activities. In rats, as in humans, the major pathway of phenytoin metabolism is hepatic *p*-hydroxylation to 4′-HPPH, and this oxidative biotransformation is exclusively mediated by the CYP2C subfamily, primarily by CYP2C6 and secondarily by CYP2C11 (Yamazaki et al 2001).

Therefore, the drug interactions of 5-FU and 5′-DFUR with phenytoin in clinical practice are explainable from the aspect of the changes in the activity of phenytoin *p*-hydroxylation and phenytoin disposition in rats.

In the present study, it was demonstrated that the activity of hepatic microsomal phenytoin *p*-hydroxylation was depressed by 30–50% 4 days after exposure to 5-FU and 5′-DFUR, even though these agents did not act as competitive inhibitors for this metabolic reaction. The depressive effect of 5′-DFUR was similar to that of 5-FU on Day 4, although relatively sustained depression was seen in 5-FU-treated rats, and there was no marked difference in the depressive potencies between repeated dosing and single dosing. Impairment of the enzyme activities was not attributable to the direct action of the agents on the enzyme molecule, as several days were required for the depression of the activity regardless of the treatment regimen. The time lag should be closely associated with the biological characteristics of 5-FU, as the exertion of antitumour activity by 5-FU is based on inhibition of DNA synthesis (Danenberg & Lockshin 1982) and perturbation of RNA processing (Ullman & Kirsch 1979), following multistep bioactivation. In examination of phenytoin pharmacokinetics in rats 4 days after exposure to 5-FU and 5′-DFUR, the CL_{tot} of phenytoin was markedly reduced, with a significant delay in the elimination process. The accessibility of phenytoin to peripheral tissues was interpreted to be unaffected owing to the lack of change in the Vd values. These results indicate that the decrease in the activity of phenytoin *p*-hydroxylation in hepatic microsomes reflected well the change in the phenytoin disposition in-vivo.

It was reported that 5-FU down-regulated the hepatic microsomal expression of several CYP species, including CYP2C and CYP3A2 (Stupans et al 1995; Afsar et al 1996) and NADPH-P450 reductase (Stupans et al 1995; Yoshisue et al 2001), with a loss of the corresponding catalytic activities. Our results were in agreement with these findings, as shown by the significant decrease in the activities of CYP2C- or CYP3A2-mediated progesterone oxidations and cytochrome c reduction after exposure of 5-FU to rats. On the other hand, a cleavage of the deoxyribose moiety is required for conversion of 5′-DFUR into 5-FU, and then 5-FU is generated to a low degree (Suzuki et al 1995). Thus, the biochemical action of 5′-DFUR is thought not to be as strong as 5-FU with respect to the potency and duration of the action. This notion was strongly supported by the small extent of the decrease in bodyweight, the P450 content, NADPH cytochrome c reductase activity and progesterone oxidation activities in rats exposed to 5′-DFUR. However, there was a similarity in the effects on the activity of phenytoin *p*-hydroxylation between 5-FU and 5′-DFUR treatments. It is noteworthy that the activities of phenytoin *p*-hydroxylation and progesterone 2α-/21-hydroxylations were found to behave differently in 5′-DFUR-treated rats, unlike 5-FU-treated rats, although their metabolic reactions share common P450 species, CYP2C6 and CYP2C11. It was demonstrated that the catalytic ability of phenytoin *p*-hydroxylation was decreased owing to a reduction in the coupling ratios of the P450 cycle in phenytoin-induced rat hepatic microsomes, despite an increase in the content of CYP2C6 and CYP2C11 being observed by immunochemical detection (Yamazaki et al 2001). A probable explanation for the disagreement in the alteration profile between phenytoin *p*-hydroxylation and progesterone hydroxylations after 5′-DFUR treatment is that 5′-DFUR affected constitutive CYP2C enzymes by modulating their conformational change and stability and/or susceptibility to various factors involved in the metabolism. This might lead to the change in their metabolic functions of phenytoin without significant impairment of the catalytic ability for other substrates, and allows us to interpret that down-regulation of hepatic CYP2C enzymes should occur during 5-FU exposure, even at a low level, which will provide clinical interest to the examination of the relevance between their disposition and pharmacological effects.

There has been no information regarding drugs that are subjected to metabolic interference by 5′-DFUR except for phenytoin. This may be owing to the specificity of the modulating effect of 5′-DFUR on hepatic CYP enzymes. According to the previous clinical reports of drug interactions between fluorouracil derivatives and phenytoin, it took at least 1 month until the onset of development of phenytoin intoxication accompanied by the elevated serum concentration after the start of fluorouracil therapy (Harada et al 1990; Wakisaka et al 1990; Hara et al 1992; Gilbar & Brodribb 2001; Konishi et al 2002). These events suggest that a latent period exists before the interaction, with either the active form or prodrugs of fluorouracil, as demonstrated by the present results.

In conclusion, the present study provided evidence that 5′-DFUR and 5-FU have depressive effects on phenytoin metabolism, and a fundamental explanation for the increased serum level of phenytoin encountered in clinical practice. Therefore, careful monitoring of the serum phenytoin concentration is recommended when given in conjunction with fluorouracil derivatives, considering that phenytoin shows non-linear kinetics within the therapeutic range (Nation et al 1990).

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