Interindividual Variability in 5-Fluorouracil Metabolism and Procarainamid N-Acetylation in Human Liver Cytosol

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We investigated the enzymatic kinetics and interindividual variability of the metabolism of 5-fluorouracil and procarainamid by human liver cytosol and/or microsomes. The Kₘ values for the 5-fluorouracil dihydropyrimidine dehydrogenase (DPD) and procarainamid N-acetyltransferase activities in pooled liver cytosol, and procarainamid hydrolysis in pooled liver microsomes were 3.9, 1670, and 969 μM, respectively, and the intrinsic clearance (Vₘ₃ₖ/Kₘ) values for these reactions were 128, 0.192, and 0.0059 μl/min/mg protein, respectively. The cytosolic activities of 5-fluorouracil metabolism and procarainamid N-acetylation ranged from 145 to 790 (469±156, mean±S.D., n=22) and <1 to 152 (52±48, n=12) pmol/min/mg protein, respectively, and the DPD activity of 5-fluorouracil was neither gender-related nor age-dependent. Procarainamid N-acetylation activities among 12 human cytosol samples were highly correlated with sulfamethazine N-acetylation activities, suggesting that procarainamid N-acetylation is catalyzed by N-acetyltransferase-2. These results suggest that the N-acetylation reaction is more important than the hydrolysis in the metabolic pathway of procarainamid, and that there are large interindividual differences in the enzyme activities towards the respective metabolic pathways of 5-fluorouracil and procarainamid in human liver.

Key words 5-fluorouracil; procarainamid; dihydropyrimidine dehydrogenase; N-acetyltransferase-2; human liver cytosol

It is well known that humans show large interindividual variations in cytochrome P450 (CYP)-catalyzed drug metabolism, and that these variations sometimes lead to different susceptibilities of humans to the pharmacological and toxicological actions of drugs, toxic chemicals, and carcinogens. For example, there are large interindividual differences in the expression levels and catalytic activities of CYP enzymes in human liver. Additionally, multiple isozymes of carboxylesterases exist in humans, and interindividual variations of the carboxylesterase activities have important clinical implications. Recently, we have reported interindividual variations in the 2-hydroxylation (CYP3A4), sulfation, and glucuronidation of ethylhexadrol in human microsomes and cytosol. On the other hand, intrinsic metabolic clearance (CLₘₚ) is calculated as a ratio of Vₘ₃ₖ to Kₘ in order to predict in vivo drug disposition. Sugiyma and colleagues proposed the prediction of in vivo metabolic clearance in experimental animals and humans from in vitro biochemical parameters such as hepatic metabolism and plasma protein binding, based on anatomically and physiologically realistic pharmacokinetic models. However, there are few reports on interindividual variations as well as enzymatic kinetics (Vₘ₃ₖ and Kₘ values) in other drug-metabolizing enzymes, including cytosolic enzymes such as dihydropyrimidine dehydrogenase (DPD) and N-acetyltransferase (NAT).

5-Fluorouracil has been one of the most widely used anticancer drugs, and it is well documented that there is wide individual variation in both the tumor response and host toxicity associated with 5-fluorouracil. In addition, marked interindividual differences in the pharmacokinetic parameters of 5-fluorouracil have been reported. The primary metabolic pathway of this drug is the reduction to 5,6-dihydrofluorouracil by DPD present in a liver cytosol fraction. After intravenous dosing to cancer patients, approximately 60—90% of the dose is excreted in urine within 24 h, primarily as α-fluoro-β-alanine, indicating that 5-fluorouracil is mainly eliminated by metabolism.

The major metabolic route of procarainamid, a class I antiarrhythmic agent, is acetylation to N-acetylprocarainamid, which is an active metabolite, and a wide variety in the amount of N-acetylprocarainamid recovered after a dose of procarainamid has been reported by different laboratories. Gibson et al. reported 7 to 34% N-acetylprocarainamid recovery in 24 h in normal volunteers after a single oral dose of procarainamid, and Karlsson et al. reported 6 to 53% recovery in 24 h in patients who were being given procarainamid chronically.

As mentioned above, there are large interindividual differences in the pharmacokinetics of 5-fluorouracil and procarainamid, which are mainly eliminated by metabolism. Therefore, we investigated the enzymatic kinetics and interindividual variability of the metabolism of 5-fluorouracil and procarainamid in human liver cytosol.

MATERIALS AND METHODS

Materials [6-14C] labeled 5-fluorouracil was purchased from Moravek Biochemicals, Inc. (Brea, CA, U.S.A.). The specific radioactivity was 1.85 GBq/mmol (50 mCi/mmol). 5-Fluorouracil, procarainamid, acetyl CoA, and NADPH were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.). All other reagents were of the highest purity commercially available.

Human Liver Microsomes and Cytosol Pooled human liver microsomes prepared from 46 individuals (20 mg protein/ml; Lot No. 0210171) were purchased from XenogenTech, LLC (Lexena, KS, U.S.A.). Human liver cytosol prepared from 12 individuals (coded HG3, HG6, HG23, HG30, HG42,
HG43, HG56, HG66, HG70, HG89, HG93, and HG112) were obtained from BD Gentest (Woburn, MA, U.S.A.), and sulfamethazine N-acetylation activities were provided with the kit (BD Gentest). Human intestinal microsomes prepared from 3 individuals (coded HIM-0012, HIM-0013, and HIM-0014) were purchased from Tissue Transformation Technologies, Inc. (Edison, NJ, U.S.A.). Human liver samples of 9 individuals (coded HL-98, HL-109, HL-113, HL-118, HL-127, HL-131, HL-132, HL-133, and HL-136) were obtained as described elsewhere. Each liver specimen was homogenized with 3 volumes of 1.15% KCl using a Teflon-glass homogenizer. The homogenate was centrifuged at 10000×g for 60 min (4°C). The supernatant was used as the cytosol. Microsomes and cytosol from human liver and intestine were stored at −80°C until use. The protein concentrations of samples prepared in our laboratories were determined by the method of Lowry et al.77 using bovine serum albumin as the standard.

**Assay for DPD Activity of 5-Fluorouracil** The DPD activity of 5-fluorouracil was measured by the method of Kanamitsu et al.13 with a minor modification. Briefly, the incubation mixture consisted of 2—100 μM [6-14C] labeled 5-fluorouracil, liver cytosol (0.5 mg protein/ml), 0.25 mM NADPH, and 35 mM potassium phosphate buffer (pH 7.4) in a final volume of 250 μl. The reaction was started by adding NADPH and the mixture was incubated at 37°C for 15 min. The reaction was terminated by adding 60 μl of 2 M KOH. After adding 15 μl of 60% perchloric acid, the mixture was centrifuged at 12000×g for 2 min. 5-Fluorouracil and α-fluoro-β-alanine, which was formed by hydrolysis of 5-fluorodihydropyrimidine by adding 2 mM KOH, were separated by thin-layer chromatography (place: Polygram CEL 300 PEI/UV254; Machery-Nagel, Germany; solvent: n-butanol/ethyl acetate/water, 4:3:2) and quantified by BAS2000 (Fujifilm, Tokyo, Japan).

**Assay for Procainamide N-Acetyltransferase Activity** The incubation mixture consisted of procainamide, liver cytosol (0.5 mg protein/ml), 1 mM acetyl CoA, and 100 mM Tris–HCl buffer (pH 7.4) in a final volume of 500 μl. After preincubation at 37°C for 3 min, the incubation mixture consisted of 0.5 to 100 mM procainamide, microsomes or cytosol (0.5 mg protein/ml) from human liver or intestine, and 100 mM Tris–HCl buffer (pH 7.4) in a final volume of 500 μl. After preincubation at 37°C for 3 min, the reaction was started by adding procainamide and the mixture was incubated at 37°C for 15 min. The reaction was terminated by adding 200 μl of acetonitrile, and the mixture was centrifuged at 12000×g for 3 min. The supernatant (20 μl) was injected into the HPLC, and the concentration of p-aminobenzoic acid was analyzed. The same HPLC system and columns as described above were used, except that the UV-detector was set at 280 nm. The mobile phase consisted of 50 mM acetic acid/methanol (92:8), and the flow rate was 1 ml/min. The retention time of p-aminobenzoic acid was 13 min.

**Data Analysis** All data were analyzed using the average of duplicate determinations. In preliminary experiments, the linearity of the reaction with incubation time and protein concentration was confirmed under each assay condition. Apparent kinetic parameters in pooled human cytosol or microsomes were estimated by fitting to Michaelis–Menten kinetics by nonlinear regression analysis (MULTI), and intrinsic metabolic clearance (CLint) was calculated as a ratio of Vmax to Km. For the calculation of CLint (μl/min/g liver), we used the following parameters: the microsomal and cytosolic protein contents in human liver were assumed to be 21.4 and 59.9 mg/g organ, respectively, as reported by Temellini et al.10 The coefficient of determination (r2) for enzyme activities was determined by linear regression analysis using a software program SAS (SAS Institute Inc., Cary, NC, U.S.A.). Statistical differences between males and females were determined by Student’s t-test. The significant level was set at p<0.05.

**RESULTS**

**5-Fluorouracil and Procainamide Metabolism by Human Liver Microsomes and Cytosol** Apparent kinetic parameters for the metabolism of 5-fluorouracil and procainamide in microsomes or cytosol from human liver are summarized in Table 1. The Km values in the 5-fluorouracil metabolism, procainamide N-acetylation, and procainamide hydrolysis were 3.9, 1670, and 969 μM, respectively, and the Vmax values were 493, 321, and 5.71 pmol/min/mg protein, respectively. The CLint (μl/min/g liver) in procainamide N-acetylation was 33-fold higher than that in procainamide hydrolysis. The activity of procainamide hydrolysis at 2 mM substrate concentration in liver cytosol or intestinal microsomes was less than 2 pmol/min/mg protein (data not shown), suggesting minor importance in the procainamide metabolism.

**Interindividual Variability of 5-Fluorouracil and Procainamide Metabolism** The interindividual variability of 5-fluorouracil metabolism in 21 liver cytosol specimens is shown in Fig. 1. The DPD activities of 5-fluorouracil ranged from 145 to 790 (469±156, mean±S.D.) pmol/min/mg protein, and there were no significant age- or gender-related differences in the activity.

The procainamide N-acetylation activities in 12 human liver cytosol samples are shown in Fig. 2. The procainamide N-acetylation activity ranged from <1 to 152 (52±48, mean±S.D.) pmol/min/mg protein, and large interindividual differences were observed. Although slight age-dependency
was observed \((r^2=0.412, p<0.05)\), the number of liver samples investigated \((n=12)\) might be insufficient for estimating the effect of age on the metabolic activity.

The correlations between procainamide N-acetylation, sulfamethazine N-acetylation, and 5-fluorouracil metabolism by human liver cytosol are shown in Fig. 3. Procainamide N-acetylation was significantly correlated with sulfamethazine N-acetylation \((n=12, r^2=0.756, p<0.001)\), whereas no significant correlation was observed between 5-fluorouracil metabolism and procainamide N-acetylation \((n=12, r^2=0.0468, p>0.05)\).

**DISCUSSION**

Although the \(K_m\) value in the procainamide N-acetylation in pooled liver cytosol was comparable with that in the hydrolysis of procainamide in pooled liver microsomes, the \(CL_{int}\) \((\mu l/min/g\) liver\) in procainamide N-acetylation was 33-fold higher than that in procainamide hydrolysis (Table 1), suggesting that the N-acetylation is the most important metabolic pathway for procainamide in human liver. Giardina *et al.*\(^{20}\) reported that 31—56%, 7—24%, and less than 0.2% of the dose were excreted as procainamide, N-acetylprocainamide, and \(p\)-aminobenzoic acid, respectively, in 24 h after a single oral dosing of \(^{14}\)C-labeled procainamide to patients with cardiac disease. Therefore, the results of the present *in vitro* study, using human liver microsomes and cytosol, are consistent with the *in vivo* clinical studies, which demonstrated that N-acetylprocainamide is a predominant metabolite after oral dosing of procainamide to humans, whereas hydrolyzed metabolites such as \(p\)-aminobenzoic acid are minor metabolites. \(^{20}\) Recently, human hepatocytes, containing both microsomal and cytosolic enzymes, were reported to be useful for the prediction of hepatic clearance of compounds, which are metabolized through several kinds of metabolic pathways, including phase I and phase II reactions. \(^{9,21}\) Further studies using hepatocytes might be required to improve the quantitative prediction of the hepatic clearance.

We have demonstrated that there is more than a 152-fold difference among individuals in the activities of procainamide N-acetylation in human liver cytosol (Fig. 2). In addition, the absence of a significant correlation between 5-
fluorouracil metabolism and procainamide N-acetylation in human liver cytosol suggests that these activities varied independently. On the other hand, correlations between procainamide N-acetylation activities and sulfamethazine N-acetylation activities were observed (Fig. 3), and these results provide support indicating that both procainamide and sulfamethazine are metabolized by N-acetyltransferase-2 (NAT2),23 whose activity is polymorphic.23 In this study, we could not evaluate the possible contribution of NAT2 polymorphisms to variability in catalytic activity of procainamide N-acetylation, because we could not obtain the genotype information of the liver donors. Correlations between acetylation phenotypes and NAT2* genotypes have been described for isoniazid, sulfamethazine, and caffeine.23 Although there are few reports describing the relationship between procainamide acetylation activity and NAT2* genotypes, Okumura et al.23 reported that the N-acetylprocainamide/procainamide ratio in urinary excretion after an oral dose of procainamide hydrochloride was the highest in NAT2*4/*4 genotype followed by NAT2*4/*6A, NAT2*4/*7B > NAT2*6A/*7B. It is possible to speculate that genetic factors, as well as environmental factors, may be major contributors.

This study showed that there are neither gender-related nor age-dependent differences in the metabolic activities of 5-fluorouracil in human liver cytosol, with a 5-fold difference among individuals (Fig. 1). It was observed in previous studies that there are marked (more than 3-fold) interindividual differences in the pharmacokinetics of 5-fluorouracil in cancer patients following intravenous or oral administration of 5-fluorouracil.12,24 In addition, 5-fluorouracil is mainly eliminated by metabolism by DPD.12,25 Recently, it has been reported that a partial deficiency of DPD in a patient results in a 2.5 fold reduction in 5-fluorouracil clearance.20 Therefore, these results suggest that, although factors, that could affect oral clearance of a drug, include absorption from the gastrointestinal tract, protein binding, intrinsic hepatic clearance, hepatic blood flow, and renal clearance, the interindividual differences of metabolism may be major contributors to the interindividual variations of 5-fluorouracil pharmacokinetics. In addition, it has been demonstrated that at least 3% of the general population could be partially deficient in DPD activity based on a population analysis of DPD activity.27 Genetic and environmental factors seem to contribute to the interindividual variation in the activity of 5-fluorouracil, but the regulation mechanism in human liver is still unknown.

In summary, this study has suggested that N-acetylation is the major metabolic pathway of procainamide in human liver, and that there are large interindividual differences in the enzyme activities towards the respective metabolic pathways of 5-fluorouracil and procainamide. It is speculated that genetic regulatory variations, including polymorphism such as DPD and N-acetyltransferase, and environmental factors may be major contributors to the interindividual variations.

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REFERENCES AND NOTES

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