Biochemical Pharmacology, Vol. 57, pp. 935–939, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(98)00375-X

SHORT COMMUNICATION

No Ethnic Difference between Caucasian and Japanese Hepatic Samples in the Expression Frequency of CYP3A5 and CYP3A7 Proteins

Tomonori Tateishi,† Minoru Watanabe,* Hitonobu Moriya,‡ Susumu Yamaguchi,‡ Tetsuo Sato§ and Shinichi Kobayashi**

*Department of Pharmacology and ‡First Department of Surgery, St. Marianna University School of Medicine, Kawasaki, Japan; and §The Biomedical Research Institute, HAB Discussion Group, Chiba, Japan

ABSTRACT. Ethnic differences in the pharmacokinetics of nifedipine, a substrate of CYP3A, and in CYP3A7 expression have been reported. The aim of the present study was to measure the protein levels of CYP3A4, CYP3A5, and CYP3A7 and nifedipine oxidation activity in hepatic microsomes from 15 Caucasian and 15 Japanese patients for comparison between the two ethnic groups. Nifedipine oxidation activity and CYP3A4 protein level were well correlated. No significant difference between Caucasian and Japanese microsomal samples was found in nifedipine oxidation activity or in the CYP3A4 protein level. CYP3A5 was detected in 6 of 15 Caucasian samples and in 5 of 15 Japanese samples, but no ethnic difference was found in either the frequency of expression or its protein level. CYP3A7 was found in 10 of 15 Caucasian samples and in 14 of 15 Japanese samples. Although the estimated CYP3A7 protein level was higher in the Japanese than in the Caucasian samples, its protein level was much lower than that of CYP3A4. These results imply that the contribution of CYP3A5 or CYP3A7 to the purported Caucasian–Japanese ethnic difference in the overall CYP3A activity seems to be small. BIOCHEM PHARMACOL **57**;8:935–939, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. nifedipine oxidation activity; CYP3A4; CYP3A5; CYP3A7; hepatic microsomes; ethnic difference; Japanese; Caucasian

There is growing evidence of ethnic differences in the activities of many drug-metabolizing enzymes for phase I and phase II reactions [1, 2]. In the CYP‖ family consisting of multiple forms of structurally and functionally distinct isoenzymes [3], the genetic polymorphisms of CYP2C19 and CYP2D6 have been particularly well investigated [4, 5], and genetic variations of other CYPs have been discovered [6].

Among the CYP subfamilies, CYP3A is the most abundant human CYP isoform, and it has a very broad substrate specificity [3]. The *in vivo* activity of CYP3A measured by nifedipine metabolism and disposition shows marked interindividual variation [7] and also suggests the possibility of an ethnic difference in activity levels [8, 9]. The human CYP3A subfamily is composed of at least three members, CYP3A4, CYP3A5, and CYP3A7 [3], with CYP3A4 being the dominant subfamily [10]. At one time, CYP3A7 was considered specific to the human fetal liver [11]. However, CYP3A7 mRNA has been reported in as many as 50% of adult liver samples [12]. CYP3A5 is expressed polymorphically, appearing in 10–25% of adult human livers [13–15]. Previous studies have involved mainly Caucasian liver samples, and little is known about the frequency of hepatic expressions of CYP3A5 and CYP3A7 and their protein levels in non-Caucasians. The spectrum of substrates and the catalytic activity of CYP3A5 are similar to those of CYP3A4; CYP3A7 shows some difference from CYP3A4 and CYP3A5 [16–18]. The expression of CYP3A5 might contribute to the ethnic differences observed in *in vivo* CYP3A activity.

Thus, we compared the protein levels of each CYP3A subfamily in human hepatic microsomes obtained from Caucasian and Japanese patients and investigated the differences in CYP3A5 and CYP3A7 expressions. We also measured nifedipine oxidation activity to study whether the presence of CYP3A5 affects the *in vitro* CYP3A activity measured by nifedipine oxidation.

MATERIALS AND METHODS

Patients and Methods

Fifteen liver samples were obtained from Japanese patients with hepatocellular carcinoma, undergoing liver resection at St. Marianna University Hospital, with their written informed consent. Hepatic tissues were procured from

† Corresponding author: Tomonori Tateishi, Department of Pharmacology, St. Marianna University School of Medicine, Kawasaki, Kanagawa 216-8511, Japan. Tel. 81–44-977–8111, Ext. 3531; FAX 81–44-975–0509; E-mail: yakuri@ma

‖ *Abbreviations*: CYP, cytochrome P450; and ECL, enhanced chemiluminescence immunoblotting.

Received 30 June 1998; accepted 19 October 1998.

TABLE 1. Patient profile, nifedipine oxidation activity, and protein level of each CYP3A subfamily

Caucasian							Japanese						
No.	Age*	Sex	Activity†	3A4‡	3A5‡	3A7‡	No.	Age*	Sex	Activity†	3A4‡	3A5‡	3A7‡
1	49	M	666	17.1	15.8	2.6	1	51	F	1080	17.7	18.7	13.5
2	71	M	1430	35.9	30.8	1.0	2	48	M	2430	72.0		8.9
3	46	M	99.9	6.4			3	70	M	2640	79.0	28.8	23.0
4	48	M	702	36.1		1.2	4	78	M	2040	62.6		40.9
5	18	M	239	5.5			5	51	F	2140	66.1	22.6	14.7
6	34	M	1470	48.3		2.2	6	56	M	2190	67.0		6.5
7	24	M	5190	165.5	68.5	14.2	7	64	M	1630	54.6		7.4
8	41	F	2080	64.6	29.8	2.2	8	36	M	1750	38.9		5.7
9	66	F	2320	62.5		2.2	9	61	M	2520	73.9		4.2
10	44	M	1420	27.0			10	65	F	1400	26.3		4.5
11	71	F	2430	75.5	18.8	1.2	11	61	M	1900	46.7	24.7	
12	25	M	1090	24.4			12	49	M	2050	59.3		15.9
13	30	M	1120	15.4	14.5		13	63	M	2100	47.5		23.5
14	49	M	3440	110.9		14.7	14	51	M	1990	45.1	23.8	16.4
15	52	F	1890	34.6		4.0	15	65	M	1820	41.0		10.6
Mean			1710	48.7	29.7	3.7	Mean			1970	53.2	23.7	11.2§
SD			1310	43.1	20.2	4.2	SD			410	17.8	3.6	8.0

All values are means of duplicate determinations. CYP3A7 levels were estimated from a comparison with microsomes of an infant aged 10 months.

*Expressed in years.

†Nifedipine oxidation activity (pmol/min/mg protein).

‡Expressed in pmol/mg protein.

§$P < 0.05$, compared with Caucasian.

portions without particular histopathological changes. No medication that affects CYP3A, such as glucocorticoids, rifampicin, macrolide antibiotics, or anticonvulsants [3], was administered within the week prior to surgery in these patients. Two Japanese patients (Japanese No. 1 and 5 in Table 1) had cirrhotic liver and two other patients (Japanese No. 9 and 14) had chronic hepatitis. Fifteen liver samples also were obtained from Caucasian transplant donors and were supplied by The National Disease Research Interchange through The Biomedical Research Institute, HAB Discussion Group. All donors except for two (Caucasian No. 7 and 8 in Table 1; No. 7 with myocardial infarction and No. 8 with unknown health problems) were patients with cerebrovascular disorders or head injuries due to a traffic accident or a gun shot. Caucasian subjects No. 8, 9, and 10 were given dexamethasone, and No. 7 was given phenytoin, in their last hospitalization. Number 7 had a history of bronchial asthma. This study was approved by the institutional review board at St. Marianna University School of Medicine. Specimens were frozen immediately after removal and kept at −80° until preparation of microsomes.

Hepatic microsomes were prepared from these samples following a method described for the preparation of rat hepatic microsomes [19]. Microsomal protein concentrations were determined by the method of Lowry *et al.* [20]. Nifedipine oxidation activity was measured according to the method of Guengerich *et al.* [21]. The expressions of CYP3A4, CYP3A5, and CYP3A7 proteins in the human liver were measured by western blot analysis. SDS–PAGE was performed according to the methods of Laemmli [22] and Guengerich *et al.* [23] using a 10.0% acrylamide gel. Aliquots of microsomal samples (2.5 μg) were subjected to SDS–PAGE, and resolved proteins were transferred to nitrocellulose sheets. Subsequently, the membranes were treated with polyclonal antibody to CYP3A2 (Daiichi Pure Chemicals) or polyclonal antibody to a synthesized peptide of CYP3A7 [24] and developed by the ECL method (Amersham). The intensities of the bands corresponding to the proteins on each membrane were measured with a densitometer (ImageQuant, Molecular Dynamics, Inc.). The protein levels of CYP3A4 and CYP3A5 were determined from the intensity of each recombinant human CYP (Gentest Corp.) used as the reference standard. In the immunoblot analysis of CYP3A7, hepatic microsomes from a 10-month-old boy were used as a positive control. Protein CYP3A7 contents were estimated based on the reaction intensity in the control microsomes to the anti-CYP3A2 antibody.

Statistical analysis for comparisons between Caucasian and Japanese samples was carried out using one-way analysis of variance and the chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Nifedipine Oxidation Activity and CYP3A4 Protein Level

Nifedipine oxidation activity and CYP3A4 protein levels (Table 1) showed marked interindividual variation and were significantly correlated ($r^2 = 0.92$, $P < 0.0001$). No significant ethnic difference was found in nifedipine oxida-

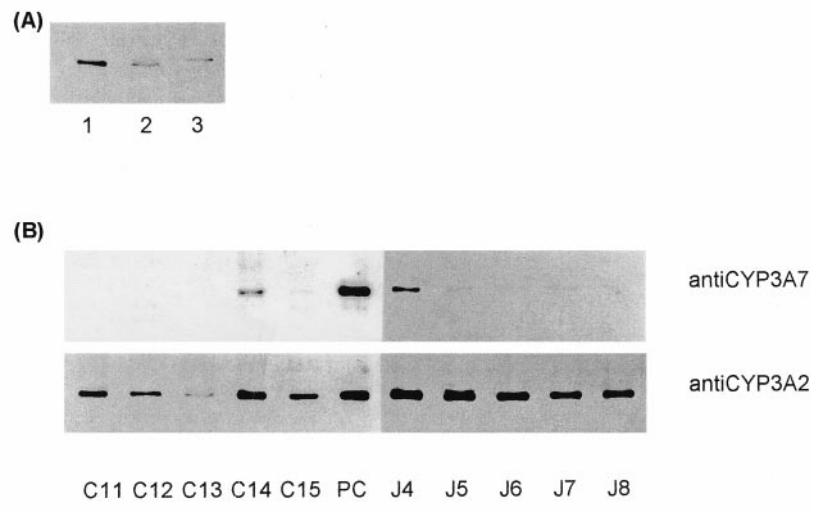

FIG. 1. Immunochemical detection of CYP3A4, CYP3A5, and CYP3A7. (A) Lanes 1, 2, and 3 show recombinant CYP3A4 (10 fmol), CYP3A4 and CYP3A5 (5 fmol each), and CYP3A5 (10 fmol), respectively. (B) In each lane, 2.5 μg of microsomal protein was applied except for C14 (1.25 μg) in the lower lanes. C and J denote a Caucasian and a Japanese liver sample, respectively. PC means a positive control, microsomes from a 10-month-old boy. The number represents the patient number in Table 1. Upper lanes were treated with anti-CYP3A7 antibody. Lower lanes were treated with anti-CYP3A2 antibody. CYP3A5 protein was identified in C11, C13, and J5.

tion activity or CYP3A4 protein levels. The mean ratio of nifedipine oxidation activity to CYP3A4 level (pmol nifedipine metabolite/pmol CYP3A4/min) was 38.4 in the Caucasian samples and 39.7 in the Japanese samples, and no significant difference was detected. Since Caucasian subject No. 7 had a history of bronchial asthma and showed the highest nifedipine oxidation activity and CYP3A4 protein level, he might have been treated with a glucocorticoid, an inducer of CYP3A protein [3]. Excluding him, nifedipine oxidation activity in the Caucasian samples was 1460 ± 920 (mean ± SD) pmol/min/mg protein, and it barely missed being significantly smaller ($P = 0.062$). Although a slightly higher level of nifedipine oxidation activity in the Japanese samples might be related to the existence of a carcinoma, Guengerich and Turvy [25] found no significant difference in the CYP3A protein level of the liver samples between the normal and metastatic cancer groups. In a previous study [10], nifedipine oxidation activity was slightly lower in Japanese samples, compared with that in Caucasian samples. No reasonable explanation can be offered for the difference. However, a relatively small number of samples might contribute to the disagreement because of extremely large interindividual variation in nifedipine oxidation activity *in vitro* [3], as well as its disposition *in vivo* [7]. The possibility of the ethnic difference in the CYP3A activity between Japanese and Caucasian populations remains to be studied.

CYP3A5 Expression Frequency and Its Protein Level

Since the molecular weight of CYP3A5 is slightly greater than that of CYP3A4 [13], these two proteins can be separated by electrophoresis (Fig. 1). CYP3A5 was detected in 6 of 15 Caucasian samples and in 5 of 15 Japanese samples, and the protein levels of CYP3A5 ranged from 14.5 to 68.5 pmol/mg protein. The results of the present study are in agreement with results in previous studies identifying the CYP3A5 protein in the liver of 10–25% of adult Caucasians [13–15] at levels ranging from 2 and 60 pmol/mg protein [15]. The frequency of hepatic CYP3A5 expression in adult Japanese patients appears to be similar to that in Caucasians, and no significant difference in its protein levels was found between the Caucasian and Japanese samples.

The mean nifedipine oxidation activity and CYP3A4 level of hepatic microsomes containing CYP3A5 (N = 11) were 2060 pmol/min/mg protein and 56.7 nmol/mg protein, respectively. The mean nifedipine oxidation activity and CYP3A4 level of hepatic microsomes not containing CYP3A5 (N = 19) were 1720 pmol/min/mg protein and 47.5 nmol/mg protein, respectively. The difference in either the activity or the protein level was not significant. Conversely, CYP3A5 was identified in 7 of 16 liver samples with higher than average nifedipine oxidation activity, and in 4 of 14 liver samples with lower than average activity. Since there was no significant difference in the frequency of CYP3A5 expression between samples with higher and lower nifedipine oxidation activity levels or CYP3A4 protein levels (chi-square test; $P = 0.4737$), CYP3A5 expression may be independent of both nifedipine oxidation activity and CYP3A4 protein level.

CYP3A7 Expression Frequency and Its Estimated Protein Level

At one time, CYP3A7 was considered specific to human fetal liver [11]. However, Schuetz *et al.* [12] reported that CYP3A7 mRNA was found in 7 of 13 adult liver samples. In the present study, we found CYP3A7 in 10 of 15 Caucasian samples, and in 14 of 15 Japanese samples. No significant correlation was observed between the intensities of CYP3A4 and CYP3A7. Therefore, it is unlikely that cross-reactivity of the anti-CYP3A7 antibody to CYP3A4 protein contributed to the high incidence of CYP3A7. Since recombinant CYP3A7 was not available, the protein level of CYP3A7 was obtained from a comparison with microsomes of a 10-month-old infant. His protein level reacting with anti-CYP3A2 antibody in microsomes was

estimated at 79 pmol/mg protein, in which some CYP3A4 protein may be included because liver microsomes from infants aged 3–12 months are reported to show 40% of the adult level of testosterone 6β-hydroxylation activity, a probe of CYP3A4 activity [26]. The CYP3A7 level of each sample was obtained from the relative intensity of its band. The results of the CYP3A7 content may be overestimated. The mean CYP3A7 level in Japanese samples was significantly higher than that in Caucasian samples. Since the Japanese liver samples were obtained from patients with hepatocellular carcinoma, hepatic tissues might have contained carcinoma cells with CYP3A7 [27]. However, the CYP3A7 protein level was much lower than the CYP3A4 protein level in both groups, and this lower protein level seemed scarcely to contribute to overall CYP3A activity, although CYP3A7 expressed in bacterial membranes shows levels of catabolic activity with some drugs similar to the levels of CYP3A4 and CYP3A5 [18].

In summary, we measured the protein level of CYP3A subfamilies expressed in human hepatic microsomes from both Caucasian and Japanese adults by the ECL method and compared them. CYP3A5 was found in 40% of Caucasian liver samples and 33% of Japanese liver samples, and no ethnic difference was found in either the frequency or the protein level. CYP3A7 was found in 67 and 93% of Caucasian and Japanese liver samples, respectively, and its protein level was much lower than that of CYP3A4. The contribution of CYP3A5 or CYP3A7 to the purported ethnic difference in the overall CYP3A activity between Japanese and Caucasians seems to be small. To confirm the results of the present study, additional studies in a greater number of samples with a similar background are desirable.

We are grateful to Dr. Tetsuya Kamataki (Hokkaido University) for the supply of anti-CYP3A7 antibody.

References

1. Wood AJJ and Zhou HH, Ethnic difference in drug disposition and responsiveness. *Clin Pharmacokinet* **20:** 350–373, 1991.
2. May DG, Genetic differences in drug disposition. *J Clin Pharmacol* **34:** 881–897, 1994.
3. Guengerich FP, Human cytochrome P450 enzymes. In: *Cytochrome P450* (Ed. Oritiz de Montellano PR), 2nd Edn, pp. 473–535. Plenum Press, New York, 1995.
4. Eichelbaum M and Gross AS, The genetic polymorphism of debrisoquine/sparteine metabolism—Clinical aspects. *Pharmacol Ther* **46:** 377–394, 1990.
5. Wilkinson GR, Guengerich FP and Branch RA, Genetic polymorphism of *S*-mephenytoin hydroxylation. *Pharmacol Ther* **36:** 773–780, 1989.
6. Daly AK, Brockmöller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, Huang JD, Idle JR, Ingelman-Sundberg M, Ishizaki T, Meyer UA, Nebert DW, Steen VM, Wolf CR and Zanger UM, Nomenclature for human CYP2D6 alleles. *Pharmacogenetics* **6:** 193–201, 1996.
7. Schellens JHM, Soons PA and Breimer DD, Lack of bimodality in nifedipine plasma kinetics in a large population of healthy subjects. *Biochem Pharmacol* **37:** 2507–2510, 1988.
8. Ahsan CH, Renwick AG, Macklin B, Challenor VF, Waller DG and George CF, Ethnic differences in the pharmacokinetics of oral nifedipine. *Br J Clin Pharmacol* **31:** 399–403, 1991.
9. Sowunmi A, Rashid TJ, Akinyinka OO and Renwick AG, Ethnic differences in nifedipine kinetics: Comparisons between Nigerians, Caucasians and South Asians. *Br J Clin Pharmacol* **40:** 489–493, 1995.
10. Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich FP, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:** 414–423, 1994.
11. Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kato R and Kanakubo Y, Purification and properties of cytochrome P-450 from homogenates of human fetal liver. *Arch Biochem Biophys* **241:** 275–280, 1985.
12. Schuetz JD, Beach DL and Guzelian PS, Selective expression of cytochrome P450 CYP3A mRNAs in embryonic and adult human liver. *Pharmacogenetics* **4:** 11–20, 1994.
13. Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fisher V, Tyndale R, Inaba T, Kalow W, Gelboin HV and Gonzalez FJ, Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J Biol Chem* **264:** 10388–10395, 1989.
14. Wrighton SA, Ring BJ, Watkins PB and Vandenbranden M, Identification of a polymorphically expressed member of the human cytochrome P-450III family. *Mol Pharmacol* **36:** 97–105, 1989.
15. Wrighton SA, Brian WR, Sari M-A, Iwasaki M, Guengerich FP, Raucy JL, Molowa DT and Vandenbranden M, Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). *Mol Pharmacol* **38:** 207–213, 1990.
16. Kitada M, Kamataki T, Itahashi K, Rikihisa T and Kanakubo Y, Significance of cytochrome P-450 (P-450 HFLa) of human fetal livers in the steroid and drug oxidations. *Biochem Pharmacol* **36:** 453–456, 1987.
17. Kitada M, Kamataki T, Itahashi K, Rikihisa T and Kanakubo Y, P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16α-hydroxylase of dehydroepiandrosterone 3-sulfate. *J Biol Chem* **262:** 13534–13537, 1987.
18. Gillam EMJ, Wunsch RM, Ueng Y-F, Shimada T, Reilly PEB, Kamataki T and Guengerich FP, Expression of cytochrome P450 3A7 in *Escherichia coli*: Effects of 5′ modification and catalytic characterization of recombinant enzyme expressed in bicistronic format with NADPH-cytochrome P450 reductase. *Arch Biochem Biophys* **346:** 81–90, 1997.
19. Nakura H, Itoh S, Kusano H, Ishizone H, Imaoka S, Funae Y, Yokoi T and Kamataki T, Decrease in the content of cytochrome P450IIE by fasting in liver microsomes of house musk shrew (*Suncus murinus*). *Biochem Pharmacol* **43:** 1907–1910, 1992.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
21. Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T and Waxman DJ, Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism inoxidative drug metabolism. *J Biol Chem* **261:** 391–407, 1986.

22. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685, 1970.
23. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits and humans using immunochemical staining coupled with SDS-polyacrylamide gel electrophoresis. *Biochemistry* **21:** 1698–1706, 1982.
24. Li Y, Yokoi T, Kitamura R, Sasaki M, Gunji M, Katsuki M and Kamataki T, Establishment of transgenic mice carring human fetus-specific CYP3A7. *Arch Biochem Biophys* **329:** 235–240, 1996.
25. Guengerich FP and Turvy CG, Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. *J Pharmacol Exp Ther* **256:** 1189–1194, 1991.
26. Lacroix D, Sonnier M, Moncion A, Cheron G and Cresteil T, Expression of CYP3A in the human liver: Evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem* **247:** 625–634, 1997.
27. Kitada M, Tsukidate K, Takeuchi J, Taneda M, Komori M, Ohi H, Itahashi K and Kamataki T, Immunochemical studies for the presence of P-450HFLa, a form of cytochrome P-450 in human fetal livers, in human hepatocellular carcinoma cells. *J Pharmacobiodyn* **12:** 341–344, 1989.