INTERSPECIES VARIATION OF CYTOCHROME P-450: ESTABLISHMENT OF CELL LINES CARRYING HUMAN ENZYMES AND APPLICATION TO TOXICOLOGY TESTING

Tetsuya Kamataki, Tsuyoshi Yokoi, Minoru Sawada and Tsutomu Sakuma,

Div. Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido Univ.
N12W6, Kita-ku, Sapporo, Hokkaido, 060 Japan

Introduction

Foreign compounds such as drugs, environmental pollutants and chemicals produced by industries are metabolized in the body to be excreated. Since resulting metabolites are in general pharmacologically and toxicologically less active than the parent compounds, the metabolic processes are regarded as a detoxication mechanism. However, there are also increasing numbers of examples showing that metabolites are more active than the parent compounds. Most carcinogens exert their genotoxicity after undergoing metabolic activation. Thus, metabolism of chemicals are important factors determining the extents of the action of chemicals.

In the present paper, we would like to show our data on 1) Interspecies homology of cytochrome P-450, a major enzyme catalyzing drug metabolism. Based on our data, we propose the efficient use of monkeys in the development of innovative drugs with low risks. 2) In vitro tool for prediction of human drug metabolism. We show data on the establishment of cell lines carrying human drug metabolizing enzymes and the application of toxicological testing.

Interspecies homology of cytochrome P-450

Unlike a wide variety of enzymes involved in the intermediate metabolism, enzymes responsible for the metabolism of foreign compounds including drugs and toxicants do not necessarily possess the same catalytic properties over animal species. The differences in the catalytic properties result in species

Table I Comparison of the Nucleotide and Deduced Amino Acid Sequences between Human and Various Animal Species

	Human	Crab-eating monkey	Marmoset	Dog	Rabbit	Hamster	Rat	Mouse
1A1	100	95	—	84	81	79	81	82
	100	94	—	82	77	77	79	80
1A2	100	—	89	84	83	80	80	79
	100	—	86	82	78	73	75	73
2C	100	96	89	73	81	79	79	—
	100	92	79	68	76	80	74	—
2D	100	—	90	—	—	76	79	74
	100	—	90	—	—	73	78	70
2E1	100	96	93	—	81	80	80	79
	100	94	89	—	79	78	80	77
3A	100	96	92	82	80	74	77	81
	100	93	87	77	74	68	71	76

Upper value: nucleotide sequence (%)
Lower value: deduced amino acid sequence (%)
— : not isolated

differences in the effectiveness and the toxicities of chemicals. While the course of development of innovative drugs, the interspecies differences have been recognized as one of the big problems since the species differences prevent the prediction of the effectiveness and the safety of the drugs in humans. Looking at genetic distance from animals to humans, one would agree with the idea that monkeys are closer to humans than are other animal species. However, no informations were available to estimate the exact nature of monkey enzymes and the exact distance between monkeys and humans with respect to drug metabolizing enzymes.

Recent development in the knowledge of molecular biology has allowed us to answer these questions. Thus, using the technique of molecular biology, we have cloned a number of cDNAs encoding cytochrome P-450 present in the liver of animals and humans and compared the sequence identity (Table I) [1]. The results indicated that monkeys were the closest species to humans. The

monkey showed about 95-96% identity to humans in the nucleotide sequences of cytochrome P-450, while other animal species including dogs, rabbits, rats and mice showed 74-84% identities, indicating additionally that the dog was not always close to humans.

Based on these data, we have proposed the most efficient use of monkeys in the development of innovative drugs as follows. 1) In an early stage of drug development, a drug under development is given to monkeys repeatedly at a challenge dose. The clinical data from the monkeys will be useful in the estimation of toxicities. 2) The kinetic data derived from the blood concentration of the drug together with analytical data of metabolites in urine and feces provide informations on the relationship between the effectiveness or toxicities and the concentration of the parent drug or metabolites. If no undesired data are obtained with a drug, then this drug can be developed with relatively low risks. 3) The monkey can be used repeatedly after 1-3 month interval to clean up the previously given drug.

In vitro systems to predict human drug metabolism

Before starting clinical studies on innovative drugs, it would be of value to obtain informations on drug metabolism in humans by in vitro studies. To obtain such informations, human livers have been used for these studies. However, there are many problems to be considered in using human liver samples. 1) Autopsy samples may not give reproducible data since degradation of enzymes may have occurred and since the data may vary dependent on the history of patients. 2) Even very fresh samples may show individual variation caused by many factors. 3) The supply of the same liver sample for considerable period of time to examine the reproducibility of the results is impossible.

Establishment of a cell line expressing human drug metabolizing enzymes and its application to toxicology testing

Mutagens/carcinogens are metabolized to chemically reactive intermediates which bind to DNA to exert their genotoxicity to cells. Thus, the enzymes involved in the metabolic activation of promutagens are essential for showing

Table II Relative Sensitivity to Heterocyclic Amines of Cell Lines Expressing CYP1A2 and NATs

Cell line	Transformed with			Relative sensitivity* to		
	CYP1A2	NAT1	NAT2	IQ	MeIQ	MeIQx
CR-68	–	–	–	1.0	1.0	1.0
A2R-5	+	–	–	1.0	1.7	1.0
CNM-4	–	+	–	1.0	1.0	1.0
ANM-13	+	+	–	1.4	1.7	2.1
CNP-16	–	–	+	1.0	1.0	1.0
ANP-25	+	–	+	380	100	100

* : Calculated from LD50 values.

genotoxicity of most chemicals. As mentioned above, there are considerable species differences in the amounts and the properties of enzymes responsible for the activation of promutagens. If cells which express human enzymes can be established, these cells will be useful in the assay which permit us to predict human genotoxicity of chemicals. For the purpose, we have introduced cDNAs encoding cytochrome P-450 and other enzymes to established cells and examined the possibility to apply the cells to toxicological testing [2-4]. In this paper, we show the results of our recent research. We introduced recombinant plasmid which carried inserts of cDNAs coding for human CYP1A2 and N-acetyltransferase (NAT) [5]. We found in previous studies that CYP1A2 was capable of activating heterocyclic amines produced by pyrolysis of proteins and amino acids rather specifically [6,7]. The NAT consists of two isozymes, mono-morphic(NAT1) and polymorphic (NAT2) NATs, and functions as an O-acetyltransferase for N-hydroxyarylamines formed by CYP1A2 from aryl-amines. A cell line which expressed CYP1A2 together with P-450 reductase activated aflatoxin B1, but not heterocyclic amines. A cell line which carried
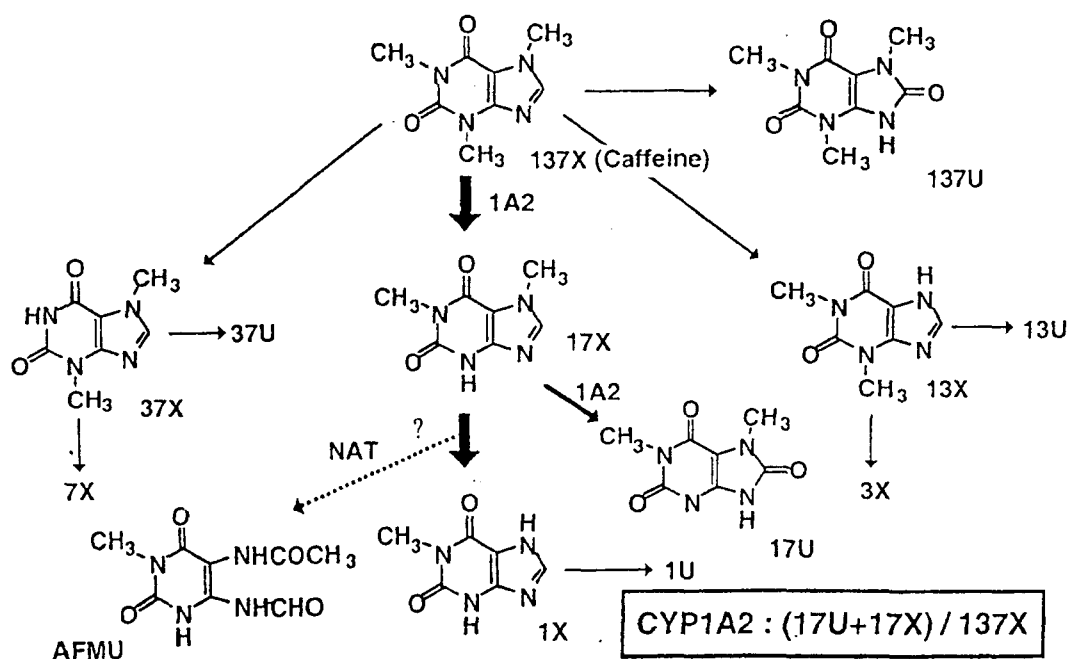
CH_3 O CH_3 137X (Caffeine) $\quad\longrightarrow\quad$ CH_3 O CH_3 =O CH_3 H 137U

$\downarrow$ 1A2

O CH_3 HN $\longrightarrow$ 37U CH_3 37X

$\downarrow$ 7X

CH_3 O CH_3 17X 1A2 CH_3 O CH_3 =O 17U

NAT ? $\cdots$

CH_3 O NHCOCH$_3$ NHCHO AFMU

CH_3 O H 1X $\longrightarrow$ 1U

CH_3 O H 13X $\longrightarrow$ 13U

$\downarrow$ 3X

CYP1A2 : (17U+17X) / 137X

Fig. 1 Pathways of Caffeine Metabolism in Humans

CYP1A2 and polymorphic NAT activated IQ and some other heterocyclic amines efficiently (Table II). However, a cell line which carried CYP1A2 and monomorphic NAT hardly activated the same heterocyclic amines. These results indicate that both CYP1A2 and polymorphic NAT are necessary to activate heterocyclic amines to mutagens, and that polymorphic expression of NAT can be a factor which affects the cancer risk by heterocyclic amine carcinogens. Recent studies have clarified that analysis of the urinary metabolites of caffeine after intake of two cups of coffee provides informations on the individual activities of CYP1A2 and polymorphic NAT (Fig. 1). Thus, to estimate the genetic polymorphism of CYP1A2 in addition to NAT in Japanese population, we performed the coffee test for 205 volunteers. The results are shown in Fig. 2. Analysis of metabolic ratios showed that there were bimodal distribution in Japanese population, indicating that genetic polymorphism was present in CYP1A2 as well as NAT. The results also showed that about 9% and 14% of Japanese were poor metabolizers of NAT and CYP1A2, respectively. Since CYP1A2 and polymorphic NAT are assumed to be factors determining
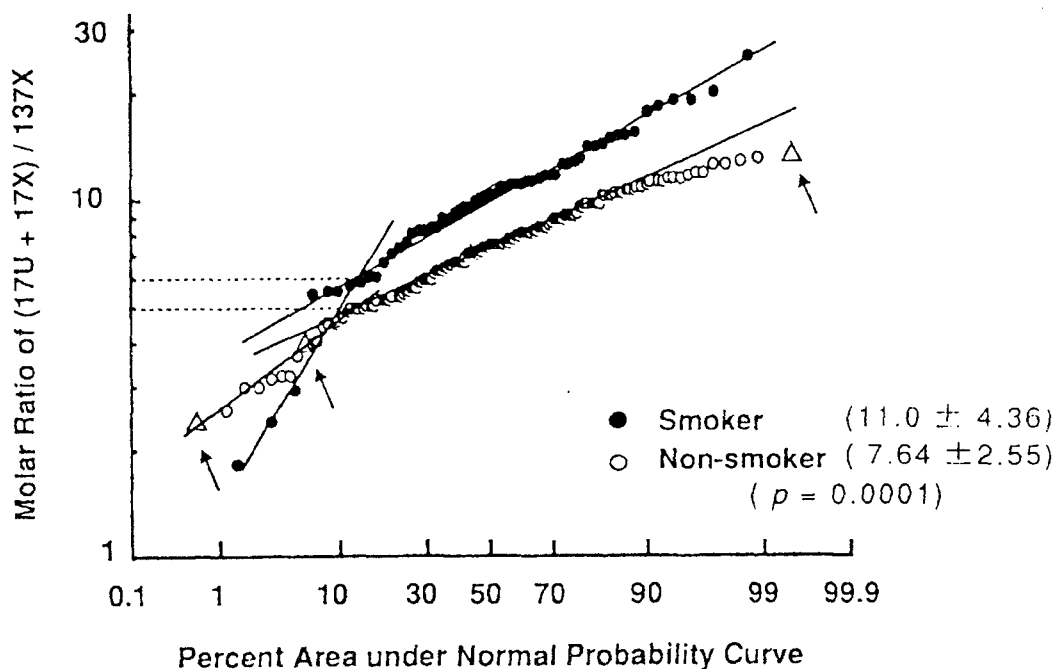
Fig. 2 Probit Analysis of CYP1A2 Activity Estimated by (17U+17X)/137X Ratio in Japanese

carcinogenic risks to heterocyclic amines derived from protein pyrolysis, it is expected that extensive metabolizers of CYP1A2 and NAT are under high risk to these amines. Supporting this idea, recent studies by Kadlubar *et al.* have demonstrated that significantly higher number of colos-rectal cancer patients were extensive metabolizers of both CYP1A2 and NAT. In order to develop a method which allows us to diagnose poor and extensive metabolizers by gene analysis, we have analyzed genomic DNA from poor and extensive metabolizers of CYP1A2. However, no gene mutations associated with the phenotypes have been found so far.

References

1) David R. Nelson, Tetsuya Kamataki, Minor J. Coon, Ronald W. Estabrook, Rene Gunsalus, Kyuichiro Okuda, David Waxman and Daniel W. Nebert: The P450 superfamily: Update on new sequences, gene mapping accession

numbers, early trivial names of enzymes, and nomenclature. DNA and Cell Biol., 12: 1-51 (1993)

2) Ryuji Kitamura, Keiko Sato, Minoru Sawada, Susumu Ito, Mitsukazu Kitada, Masayuki Komori and Tetsuya Kamataki: Stable expression of cytochrome P-450IIIA7 cDNA in human breast cancer cell line MCF-7 and its application to cytotoxicity testing. Archs. Biochem. Biophys., 292: 136-140 (1992)

3) Minoru Sawada, Ryuji Kitamura and Tetsuya Kamataki: Stable expression of monkey cytochrome P-450IA1 cDNA in Chinese hamster CHL cells and its application for detection of mutagenicity of aflatoxin B_1. Mut. Res., 265:23-29 (1992)

4) Minoru Sawada, Ryuji Kitamura, Satoru Ohgiya and Tetsuya Kamataki: Stable expression of mouse NADPH-cytochrome P450 reductase and monkey P4501A1 cDNAs in Chinese hamster cells: Establishment of cell lines highly sensitive to aflatoxin B1. Archs. Biochem. Biophys., 300: 164-168 (1993)

5) Yoshiki Yanagawa, Minoru Sawada, Takeo Deguchi, Frank J. Gonzalez and Tetsuya Kamataki: Stable expression of human CYP1A2 and N-acetyl-transferases: Mutagenic activation of 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) and 2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline (MeIQx). Submitted for publication (1994)

6) Tetsuya Kamataki, Kaori Maeda, Yasushi Yamazoe, Naomi Matsuda, Kenji Ishii and Ryuichi Kato: A high spin form of cytochrome P-450 highly purified from PCB-treated rats: Catalytic characterization and immunological quantitation in liver microsomes. Mol. Pharmacol., 24: 146-155 (1983)

7) Yasushi Yamazoe, Miki Shimada, Tetsuya Kamataki and Ryuichi Kato: Microsomal activation of 2-amino-3-methylimidazo[4,5-f]quinoline, a pyrolysate of sardine and beef extract, to a mutagenic intermediate. Cancer Res., 43: 5768-5774 (1983)