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Review

The role of polymorphic cytochrome P450 enzymes in drug design, development and drug interactions with a special emphasis on phenotyping

Emel Arınç*

Biochemistry Graduate Programme and Department of Biological Sciences, Middle East Technical University, Ankara 06531, Turkey

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ABSTRACT

Inhibitors of some cytochrome P450s (CYPs) are used to design target-specific drugs. CYPs belonging to families 1–4 play important roles in drug metabolism and therapeutics. Some isozymes of CYPs also activate pro-carcinogens into their carcinogenic forms. Approximately 40–50% human CYP-dependent drug metabolism is carried out by polymorphic CYPs resulting in therapeutic failure and adverse reactions. Phenotyping of CYPs involved in the metabolism of a drug is important to understand the potential of clinical interactions and to predict the possible individual variations due to genetic polymorphisms of certain CYPs.

Contents

1. Introduction

Cytochrome P450 is a class of heme-thiolate proteins that function as the terminal oxidases in NADPH-dependent electron transport pathways. In endoplasmic reticulum, FAD- and FMN-containing flavoprotein, NADPH-dependent cytochrome P450 reductase, directly catalyzes electron transfer from NADPH to P450. More than 8000 *CYP* genes are identified in living organisms and 18 families are described for humans. CYPs in families 1–4 are responsible for about 75–80% of all phase I-dependent metabolism of clinically used drugs and of thousands of xenobiotics. They also play a crucial role in the synthesis and metabolism of a variety of physiologically active compounds including steroid hormones, bile acids, vitamin D, thromboxane and prostaglandins [1].

2. Cytochrome P450s as drug targets

CYPs have been shown to have an increasingly important role during drug design and development. Inhibitors of specific CYPs are used to design target-specific rational drugs. One of the long-standing targets is aromatase enzyme, CYP19A1. Aromatase plays a pivotal role in the synthesis of estrogens and catalyzes the conversion of androstedione to estrone, and testosterone to estradiole. CYP19A1 inhibitors such as fadrozole, anastrozole, and letrozole are used as potentially useful drugs for the control of estrogen-dependent mammary tumors [2,3]. Since excessive androgens can induce development of prostate cancer, inhibitors of CYP17A1 (catalyzing androgen production) such as ketoconozole and inhibitors

* Tel.: +90 312 2105192; fax: +90 312 2107976.
E-mail address: earinc@metu.edu.tr.

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of CYP51A1, lanosterol 14alpha demethylase, are used as drugs in the treatment of prostate cancer and systemic mycoses, respectively [4].

3. Cytochrome P450s in drug metabolism, interactions and chemical carcinogenesis

While most of the human CYPs, CYP1A2, CYP2B6, CYP2A6, CYP2C8, CYP2C9, CYP19, CYP2E1, CYP2D6 and CYP3A4, are primarily located in the liver [5], some isozymes of CYPs (CYP1A1, CYP1B1, CYP2S1, CYP4A1 and CYP4A4) are expressed in extrahepatic tissues [5–8]. About 50% of the clinically used drugs are metabolized by CYP3A4. This is followed by CYP2D6 (25%), CYP2C9/19 (20%). The remaining drug metabolism is mainly catalyzed by CYP2A6, CYP1A2, and CYP2B6 [5].

The major concern of the drug industry and medical sciences today is the extensive interindividual variation in drug metabolism which results in therapeutic failure, unpredictable drug response, and toxicity. The variation observed in drug metabolism is mainly due to induction or inhibition of these enzymes resulting from multiple drug therapies or environmental factors, disease states and genetic polymorphisms [5,9–11]. Drug–drug interactions during multiple drug therapy result in competitive inhibition of CYP enzymes responsible for elimination of the drug. Such an interaction leads to increased plasma levels of drugs with the potential for concomitant toxic effects. Induction of CYPs by drugs lowers the plasma levels, and pharmacological response is reduced.

Persistent organic pollutants such as aromatic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PCBs) specifically induce CYP1A1 through Ah receptor mediated mechanism. At the same time, CYP1A1 mostly converts PAH- and PCB-type precarcinogens to their epoxides or other oxygenated metabolites. These metabolites, in turn bind DNA, covalently forming DNA-adducts which may cause cancer in the years to come. A 10- to 100-fold induction of CYP1A1 was observed [10]. On the other hand, CYP1A2 activates many precarcinogenic arylamines and amides present in food and cigarette smoke. Scientists are now regarding the potential of a new drug candidate to induce CYP1A as an indicator of likely carcinogenicity [12], and questioning the candidate drug's eligibility for further drug development studies. Among the CYPs, CYP2E1 the classical ethanol-inducible CYP isozyme has also been shown to convert low molecular weight pro-carcinogens such as *N*-nitrosodimethylamine, benzene and acrylamide into carcinogenic forms [11,13,14].

4. Pharmacogenetics

Pharmacogenetics investigates interindividual differences in response to a drug as a result of genetic mutations. The major concern of medical sciences and drug industry today is the genetic polymorphism observed in the genes encoding drug transport and target proteins (receptors), and in Phase I and Phase II drug metabolizing enzymes, which results in therapeutic failure, adverse drug reactions and toxicity [5,9,14]. Pharmacogenetic knowledge is important in adjustment of a drug dose which is specific for an individual.

5. Pharmacogenetics of cytochrome P450s

Approximately 40–50% of human CYP-dependent drug metabolism is carried out by polymorphic CYPs. Mutations in *CYP* genes result in enzyme variants with higher, lower, or no activity or may lead to a total absence of enzyme. Genetic polymorphism has been divided into three classes of phenotypes based on the extent of drug metabolism in the populations; poor metabolizers (PM), have mutation and/or deletions in both alleles for phenotypic expression; extensive metabolizers (EM), characteristic of normal population, carry two functional genes; ultrarapid metabolizers (UM) carry duplicated, multiduplicated or amplified genes. Most of the CYP genes show polymorphism to some extent. Among them, CYP2C9, CYPC19, and CYP2D6 have been shown to be highly polymorphic. These CYPs metabolize about 40–45% of drugs present in the market [5]. As a result of ultrarapid metabolism of a drug, the concentration of a drug in blood decreases resulting in therapeutic failure. Increased concentration of a drug observed in slow metabolizers results in high pharmacological action and toxic effect. CYP2D6 is responsible for the metabolism and therapeutic effect of many drugs including anti-depressants, neuroleptics and anticancer drugs such as tamoxifen [5,9,15,16]. If a drug, like tamoxifen, requires bioactivation for its therapeutic effect, slow metabolizers decrease the formation of an active drug resulting in reduced chemotherapeutic effect [15,16]. Tamoxifen, a selective estrogen receptor modulator (SERM), is widely used for the treatment of all stages of hormone-receptor positive breast cancer and for the prevention of breast cancer in women at high risk. However, the efficacy of tamoxifen treatment for breast cancer treatment varies widely among individuals, and about 35% of the patients with estrogen positive tumors do not respond to tamoxifen therapy. Tamoxifen is metabolized to its pharmacologically active metabolite, endoxifen (*N*-desmethyl 4-hydroxy-tamoxifen), via 4-hydroxylation of *N*-desmethyltamoxifen by the CYP2D6 enzyme. The steady-state endoxifen plasma concentrations during tamoxifen treatment were found to be substantially reduced in women that carry *CYP2D6* genetic variants especially *CYP2D6*4* (poor metabolizer, see Table 1). When patients took tamoxifen concomitantly with anti-depressant drugs, such as paroxentine (a potent inhibitor of CYP2D6), the plasma concentration of endoxifen was also reduced substantially [15,16]. It is not unusual to find a 10-fold or as much as a 50-fold difference in the rate of drug metabolism catalyzed by CYP2D6 variants among patients.

6. Ethnic differences

It is well established that relative distribution of variant alleles for the drug-metabolizing enzymes differs markedly between ethnic groups [5,9,14,17–21]. The prevalence of major CYP2A6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 variants in different ethnic groups is shown in Table 1. The frequency of CYP2D6*4 gene is 12–21% among Caucasians and 11–21% in Turkish population which is also Caucasian in ethnic origin. While the carriers of this gene in Asians and Black Africans are 1% and 2%, respectively. Similarly, frequency of CYP2C9*2 is reported as 10% in Turkish population and 8–13% in other Caucasians and none in Asians. Ethnic differences are also noted in the CYP2E1 polymorphism, in which the prevalence of CYP2E1*5 allele is 25–36% in Eastern Asian population, whereas the frequencies are significantly lower in Caucasians (Table 1) [14,20]. The frequency of CYP*6 also shows substantial ethnic differences with 2–8% occurring in Caucasians and up to 30% in Eastern Asians [14,20].

7. Phenotyping

It is well known that several CYPs are present in a given organ. Multiple forms of CYPs including the highly polymorphic forms show different but overlapping substrate specificities and reactivities toward these compounds. Besides, the balance between detoxification and bioactivation of a compound in a particular species or organ is highly dependent on the relative amounts and/or activation of different forms of P450s that are expressed. Thus, identification of individual CYPs (phenotyping) for forma-

Table 1
Ethnic group differences among human polymorphic cytochrome P450 enzymes and their major variant alleles.

Enzyme	Major variant alleles	Mutation	Consequences for enzyme function	Allele frequencies (%)			
				Turkish	Caucasians[a]	Asians[a]	Black Africans[a]
CYP2A6	CYP2A6*2	Leu160His	Inactive enzyme		1–3	0	ND
	CYP2A6del	Gene deletion	No enzyme		1	15	ND
CYP2C9	CYP2C9*2	Arg144Cys	Reduced affinity for P450 reductase	10[b]	8–13	0	ND
	CYP2C9*3	Ile359Leu	Altered substrate specificty	9–10[b]	6–9	2–3	ND
CYP2C19	CYP2C19*2	Abberant splice site	Inactive enzyme	12[c]	13	23–32	13
	CYP2C19*3	Premature stop codon	Inactive enzyme	0.4[c]	0	6–10	ND
CYP2D6	CYP2D6*2 × N	Gene duplication or multiduplication	Increased enzyme activity		1–5	0–2	2
	CYP2D6*4	Defective splicing	Inactive enzyme	11–21[c,d]	12–21	1	2
	CYP2D6*5	Gene deletion	No enzyme		2–7	6	4
	CYP2D6*10	Pro37Ser, Ser486Thr	Unstable enzyme	6[d]	1–2	51	6
	CYP2D6*17	Thr107Ile, Arg296Cys, Ser486Thr	Reduced affinity for substrates		0	ND	34
CYP2E1	CYP2E1*5B	G-1293C/C-1053T	Altered expression	1.9[e]	2–8[e]	25–36[e]	ND
	CYP2E1*6	T7632A	–	8.2[e]	8[e]	30[e]	ND
	CYP2E1*7B	G-71T	Altered expression	6.8[e]	4–6[e]	ND	ND

[a] Data compiled from Ref. [9].
[b] Data compiled from Ref. [17].
[c] Data compiled from Ref. [18].
[d] Data compiled from Ref. [19].
[e] Data compiled from Refs. [14,20].

tion of specific metabolites of drugs, pro-carcinogens as well as their distribution in a particular species or an organ is the major task for the researchers. To do this successfully, more than one approach should be used. Individually purified and/or recombinant CYP isozymes are valuable tools for evaluating the intrinsic ability of each individual isozyme to metabolize a drug. In addition, kinetic parameters of substrate probes, studies with highly selective chemical inhibitors and/or antibodies on the metabolism of the new drug enable evaluation of the relative contribution of a particular CYP isozyme [8,22,23]. Knowledge on the specific CYP isozymes that are involved in the metabolism of a new drug is important to understand the potential of clinical drug interactions. The drugs metabolized by the same CYP isozyme such as CYP3A4, when taken in 0–2 h intervals, cause drug interactions due to competitive inhibition.

8. Conclusion

CYPs play important roles in drug metabolism, chemical carcinogenesis and therapeutics. In addition, inhibitors of specific CYPs are used to design target-specific rational drugs. Genetic polymorphisms observed in drug-metabolizing CYPs result in therapeutic failure, adverse drug reactions and toxicity. The major polymorphisms that have clinical consequences are those related to the metabolism of drugs by CYP9/19 and CYP2D6. This information is currently being used by the drug industry during drug development. Candidate drugs that are selectively metabolized by polymorphic CYPs are often dropped early in the drug development process. Thus, it is expected to have fewer problems with polymorphic enzymes during drug therapy in future.

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References

[1] E. Arınç, A. Sen, Comp. Biochem. Physiol. 104B (1993) 133–139.
[2] A.Y.H. Lu, E. Arınç, J.B. Schenkman, E. Hodgson (Eds.), Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health, Springer-Verlag, Heidelberg, 1995, pp. 503–513.
[3] J.B. Manders, W.J. Gradishar, Breast Cancer 12 (2005) 62–72.
[4] H. Vanden Bossche, P. Marichal, G. Willenses, P.A.J. Janssen, E. Arınç, J.B. Schenkman, E. Hodgson (Eds.), Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes, Plenum Press, New York, 1989, pp. 345–363.
[5] M. Ingelman-Sundberg, C. Rodriguez-Antona, Phil. Trans. R. Soc. 360 (2005) 1563–1570.
[6] E. Arınç, R.M. Philpot, J. Biol. Chem. 251 (1976) 3213–3220.
[7] O. Adali, E. Arınç, Int. J. Biochem. 22 (1990) 1433–1444.
[8] E. Arınç, J.B. Schenkman, H. Greim (Eds.), Handbook of Experimental Pharmacology Cytochrome P450, vol. 105, Springer-Verlag, Heidelberg, 1993, pp. 373–386.
[9] M. Ingelman-Sundberg, M. Oscarson, R.A. McLellan, Trends Pharmacol. Sci. 20 (1999) 342–349.
[10] E. Arınç, A. Şen, B. Bozcaarmutlu, Pure Appl. Chem. 72 (2000) 985–994.
[11] E. Arınç, S. Arslan, O. Adali, Arch. Toxicol. 79 (2005) 427–433.
[12] C. Ionnides, Drug Metab. Rev. 25 (1993) 483–501.
[13] C.S. Yang, J.Y. Hong, E. Arınç, J.B. Schenkman, E. Hodgson (Eds.), Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health, Springer-Verlag, Heidelberg, 1995, pp. 181–192.
[14] G. Ulusoy, E. Arınç, O. Adali, Arch. Toxicol. 81 (2007) 711–718.
[15] Y. Jin, Z. Desta, V. Stearns, B. Ward, H. Ho, L. Kyung-Hoon, T. Skaar, A.M. Storniolo, L. Lang, A. Araba, R. Blanchard, A. Nugyen, L. Ullmer, J. Hayden, S. Lemler, R.M. Weinshilboum, J.M. Rae, D.F. Hayes, D.A. Flockhard, J. Natl. Cancer Inst. 97 (2005) 30–39.
[16] Z. Desta, D.A. Flockhart, J. Clin. Pharmacol. 25 (2007) 5147–5149.
[17] A.S. Aynacioglu, J. Brockmoller, S. Bauer, C. Sache, P. Guzelbey, Z. Ongen, M. Nacak, I. Roots, Br. J. Clin. Pharmacol. 48 (1999) 409–415.
[18] A.S. Aynacioglu, C. Sachse, A. Bozkurt, S. Kortunay, M. Nacak, T. Schroder, S.O. Kayaalp, I. Roots, J. Brockmoller, Clin. Pharmacol. Ther. 66 (1999) 185–192.
[19] A. Koseler, Y.O. Ilkol, I.H. Ulus, Pharmacology 79 (2007) 203–206.
[20] G. Ulusoy, O. Adali, T.B. Tümer, G. Şahin, S. Gozdaşoglu, E. Arınç, Oncology 72 (2007) 125–131.
[21] T.B. Tümer, G. Ulusoy, O. Adali, G. Şahin, S. Gozdaşoglu, E. Arınç, Am. J. Hematol. 82 (2007) 906–910.
[22] E. Arınç, A. Bozcaarmutlu, J. Biochem, Mol. Toxicol. 17 (2003) 169–176.
[23] T. Akutsu, K. Kobayashi, K. Sakurada, H. Ikegaya, T. Furihata, K. Chiba, Drug Metab. Dispos. 35 (2007) 72–78.