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Emerging New Technologies in Pharamcogenomics: Rapid SNP detection, molecular dynamic simulation, and QSAR analysis methods to validate clinically important genetic variants of human ABC Transporter ABCB1 (P-gp/MDR1)

Toshihisa Ishikawa [a,b,*], Aki Sakurai [a,b], Hiroyuki Hirano [a], Alexander Lezhava [b], Minoru Sakurai [a], Yoshihide Hayashizaki [b]

[a] Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, 225-8501, Japan
[b] Omics Science Center, RIKEN Yokohama Institute, Yokohama 230-0045, Japan

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ABSTRACT

Pharmacogenomics, the study of the influence of genetic factors on drug action, is increasingly important for predicting pharmacokinetics profiles and/or adverse reactions to drugs. Drug transporters as well as drug-metabolism play pivotal roles in determining the pharmacokinetic profiles of drugs and, by extension, their overall pharmacological effects. There are an increasing number of reports addressing genetic polymorphisms of drug transporters. A key requirement for the development of individualized medicine or personalized therapy is the ability to rapidly and conveniently test patients for genetic polymorphisms and/or mutations. We have recently developed a rapid and cost-effective method for single nucleotide polymorphism (SNP) detection, named Smart Amplification Process 2 (SmartAmp2), which enables us to detect genetic polymorphisms or mutations in 30 to 45 min under isothermal conditions without DNA isolation and PCR amplification. Furthermore, high-speed functional screening, quantitative structure-activity relationship (QSAR) analysis, and molecular dynamic (MD) simulation methods have been developed to study the substrate specificity of ABC transporters and to evaluate the effect of genetic polymorphisms on their function and substrate specificity. These methods would provide powerful and practical tools for screening synthetic and natural compounds, and the deduced data can be applied to the molecular design of new drugs. This review addresses such new methods for validating genetic polymorphisms of human ABC transporter ABCB1 (P-gp/MDR1) which is critically involved in the pharmacokinetics of drugs.

Contents

Abbreviations: ABC, ATP-binding cassette; BBB, blood brain barrier; MD simulation, molecular dynamic simulation; MDR, multidrug resistance; P-gp, P-glycoprotein; QSAR, quantitative structure-activity relationship; SmartAmp2, Smart Amplification Process 2; SNP, single nucleotide polymorphism.

* Corresponding author. RIKEN Yokohama Institute, Omics Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan. Tel.: +81 45 503 9222; fax: +81 45 503 9216.
E-mail address: toshi-i@gsc.riken.jp (T. Ishikawa).

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1. Introduction

Clinical observation beginning in the 1950 s suggested that individuals exhibit differences in their response to drugs and that these variations could be inherited (Evans & McLeod, 2003; Kalow, Meyer & Tyndale, 2001). Medical practice based on population responses did not reflect the best treatment for an individual (Haselden & Nicholls, 2006). Numerous studies made during the past decades clearly established that genetic factors influence the heterogeneity of individual responses to medications with respect to both toxicity and efficacy (Kalow et al., 2002). Polymorphisms in the human genome contribute to wide variations in how individuals respond to medications, either by changing the pharmacokinetics (absorption, distribution, metabolism, and elimination) of drugs or by altering the cellular response to therapeutic agents.

Metabolic systems for xenobiotics including drugs are widely referred to as phase I and II systems, where phase I includes oxidation of xenobiotics and phase II deals with the conjugation of phase I products. Oxidative metabolism in the phase I system is mediated by cytochrome P-450 (CYP) or flavin mixed-function oxidases. Some of activated xenobiotics can interact with DNA and/or proteins in cells to cause toxic effects. In the phase II system, on the other hand, activated hydrophobic xenobiotics are converted into hydrophilic forms via conjugation reactions with glutathione, sulfate, or glucuronide. This phase II metabolism is regarded as the detoxification process for xenobiotics. In some cases, however, the phase II system is a critical step in the formation of genotoxic electrophiles. Furthurmore, accumulation of the resulting metabolites in cells can lead to a decrease in the detoxification activity of the phase II system. Genetic polymorphisms of drug metabolizing enzymes in phase I and II systems and their contribution to pharmacokinetics and adverse effects have been well studied. In 1992, on the other hand, Ishikawa proposed a concept for a “phase III” detoxification system by emphasizing the biological importance of ATP-dependent export pumps (Ishikawa, 1992; Toyoda et al., 2008). Since that time, many transporter genes have been discovered. Recent progress has been made in understanding the role of membrane transporters in drug safety and efficacy. In particular, over 400 membrane transporters in two major superfamilies, i.e., the ATP-binding cassette (ABC) transporters and the solute carriers (SLC), have been identified in the human genome. Many of SLC transporters have been reported to play critical roles in influx of drugs into cells as well as in exporting drugs and their metabolites.

ABC transporters form one of the largest protein families encoded in the human genome (Dean, Rzhetsky & Allikmets, 2001; Holland, Cole, Kuchler & Higgins, 2003). Hitherto more than 48 human ABC transporter genes have been identified and sequenced (Klein, Sarkadi & Váradi, 1999). It has been reported that mutations of ABC transporter genes are causative of several genetic disorders in humans (Dean et al., 2001). Many of the human ABC proteins are involved in membrane transport of drugs, xenobiotics, endogenous substances, or ions, thereby exhibiting a wide spectrum of biological functions (Schinkel & Jonker, 2003). Based on the arrangement of molecular structure components, i.e., nucleotide binding domains and topologies of transmembrane domains, the hitherto reported human ABC transporters have been classified into 7 different sub-families (A to G) (Klein et al., 1999; Borst and Oude Elferink, 2002; Ishikawa, 2003). Several ABC transporters, including ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, and ABCG2, are considered to be major players in the phase III detoxification system (Ishikawa, 1992; Borst & Oude Elferink, 2002; Schinkel & Jonker., 2003; Toyoda et al., 2008). Expression of those transporters is regulated by transcriptional activation and/or protein degradation.

There are an enormous number of literatures focusing on the interaction of drugs and their metabolites with drug transporters. In drug discovery and development in the pharmaceutical industry, increasing attention has been paid to drug transporters expressed in epithelia of the intestine, liver and kidney, and the endothelium of the blood brain barrier (BBB) as well as in drug-resistant cancer cells. In this context, the FDA Critical Path Transporter Workshop has recently been held in Bethesda, MD to prepare a whitepaper. The scope of the whitepaper is to (i) identify transporters, which, based on current knowledge, are the most important determinants of pharmacokinetics; (ii) discuss methodologies to characterize drug-transporter interactions in *in vitro* and *in vivo* studies; and (iii) propose recommendations important for drug development scientists in guiding pre-clinical and clinical studies of transporter-mediated drug interactions.

2. Genetic polymorphisms in human ABCB1 (P-glycoprotein/MDR1) gene

Pharmacogenomics of drug transporters would provide new knowledge and practical tools for personalized medicine (Ishikawa et al., 2004). Human ABCB1 (P-glycoprotein or MDR1) was first identified because of its overexpression in cultured cancer cells associated with an acquired cross-resistance to multiple anticancer drugs (Ambudkar, Lelong, Zhang & Cardarelli, 1998; Gottesman & Ling, 2006; Ambudkar et al., 2006). ABCB1 is expressed not only in cancer cells but also in many normal tissues. For example, it is located in the apical domain of the enterocytes of the gastrointestinal tract (jejunum and duodenum) and limits the uptake and absorption of drugs and other substrates from the intestine into the systemic circulation by excreting substrates into the gastrointestinal tract (Lown et al., 1997; Masuda et al., 2000). In addition, ABCB1 is expressed in the endothelial cells lining the small vessels of the human cortex, in which the transporter appears to be concentrated within the luminal cellular compartment. The expression of ABCB1 on the luminal membrane of capillary endothelial cells of the brain restricts drug distribution into the central nervous system (Schinkel et al., 1994; Virgintino et al., 2002). This function of ABCB1 appears to be very important for protecting the central nervous system from attack by toxic compounds. On the other hand, distribution of certain drugs into the central nervous system is limited by the function of ABCB1 expressed in the blood brain barrier (BBB).

The statistical analysis of ABCB1 genetic variation data indicates that there is considerable nucleotide diversity in this gene (Kerb, Hoffmeyer & Brinkmann, 2001; Kroetz et al., 2003; Leaberman et al., 2003). Hitherto, more than 50 SNPs and several insertion/deletion polymorphisms in the ABCB1 gene have been reported. Preclinical and clinical studies have provided evidence for naturally occurring polymorphisms in ABCB1 and their effects on drug absorption, distribution, and elimination (Hoffmeyer et al., 2000; Kim et al., 2001; Horinouchi et al., 2002; Itoda et al., 2002; Johne et al., 2002; Macphee et al., 2002; Saito et al., 2002; Siegmund et al., 2002; Kafka et al., 2003; Kroetz et al., 2003; Saito et al., 2003; Sakaeda et al., 2003; Marzolini et al., 2004; Ozawa et al., 2004; Uwai et al., 2004; Allabi et al., 2005; Sakurai et al., 2005; Sakaeda, 2005). It is possible that synonymous or promoter region variants can influence the expression level of ABCB1 (Ishikawa et al., 2004), whereas a polymorphism that affects ABCB1 activity at the protein level most probably may be an amino acid changing non-synonymous variant. Amino acid changes may alter key domains necessary for substrate binding, ATP hydrolysis, or protein folding.

Hoffmeyer et al. (2000) first reported multiple polymorphisms in the ABCB1 gene. One of those mutations in particular, a C-to-T variant at position 3435 in exon 26 of the ABCB1 gene, was correlated with ABCB1 expression and function, whereas an association of the 3435C>T polymorphism with ABCB1 protein expression and function remains controversial. It has been suggested that the 2677G>T/3435C>T haplotype is of clinical importance (Horinouchi et al., 2002; Johne et al., 2002; Kroetz, et al., 2003; Green, Soderkvist, Rosenberg, Horvath &