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Validation of in vitro cell models used in drug metabolism and transport studies; genotyping of cytochrome P450, phase II enzymes and drug transporter polymorphisms in the human hepatoma (HepG2), ovarian carcinoma (IGROV-1) and colon carcinoma (CaCo-2, LS180) cell lines

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Abstract

Human cell lines are often used for in vitro biotransformation and transport studies of drugs. In vivo, genetic polymorphisms have been identified in drug-metabolizing enzymes and ABC-drug transporters leading to altered enzyme activity, or a change in the inducibility of these enzymes. These genetic polymorphisms could also influence the outcome of studies using human cell lines. Therefore, the aim of our study was to pharmacogenotype four cell lines frequently used in drug metabolism and transport studies, HepG2, IGROV-1, CaCo-2 and LS180, for genetic polymorphisms in biotransformation enzymes and drug transporters.

The results indicate that, despite the presence of some genetic polymorphisms, no real effects influencing the activity of metabolizing enzymes or drug transporters in the investigated cell lines are expected. However, this characterization will be an aid in the interpretation of the results of biotransformation and transport studies using these in vitro cell models.
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Introduction

Variability in drug metabolism and drug transport plays an important role in human drug toxicology and therapeutic efficacy (Wormhoudt et al., 1999). An important aspect in the development of new drugs is therefore the elucidation of drug metabolism pathways, assessment of the pharmacological and toxicological activity of formed metabolites compared to the parent drug, identification of the metabolic enzymes involved in the biotransformation and the elucidation of the role of drug transport in the uptake and excretion of a drug.

Biotransformation pathways can be divided into two categories; phase I (oxidation, reduction and hydrolysis) and phase II (conjugation) reactions (Derelanko and Hollinger, 1995; Crommentuyn et al., 1998). The cytochrome P450 (CYP) superfamily is the largest and most important group of the phase I enzymes and the CYP3A, CYP2D and

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CYP2C subfamilies are responsible for, respectively, 50%, 25 and 20% of the biotransformation of all drugs (Smith and Jones, 1991; Wrighton and Stevens, 1992; Lu, 1996). Major enzymes involved in phase II reactions are uridine diphosphoglucuronosyl transferase (UGT), sulfotransferase (SULT), *N*-acetyltransferase (NAT) and glutathione-*S*-transferase (GST) (Lu, 1996; Range et al., 1996; Ritter, 2000).

The drug transporters of the ATP binding cassette (ABC)-containing family of proteins have a well-established effect on the pharmacokinetics of many clinically relevant drugs. These transporters influence the oral bioavailability and the hepatobiliary, intestinal and urinary excretion of drugs and their metabolites. The most important members of the ABC-family are P-glycoprotein (P-gp, MDR1), multidrug resistance protein 2 (MRP2, ABCC2) and the breast cancer resistance protein (BCRP, ABCG2) (reviewed by Schinkel and Jonker, 2003).

Several strategies have been developed to study human drug metabolism and active transport of drugs in vitro, like the use of transformed cell lines (reviewed by Brandon et al., 2003; Plant, 2004; Varma et al., 2003; Braun et al., 2000). Unfortunately, preclincial model systems used for assessment of human drug metabolism and drug transport poorly predict biotransformation and elimination in man. A number of factors may explain this discrepancy. Expression of drug-metabolizing enzymes and drug transporters in transformed cell lines is often low and variable, as in the often applied HepG2 human hepatoma cell system (Knasmuller et al., 1998; Brandon et al., 2003; Wilkening and Bader, 2003). Furthermore, genetic variability in these enzyme and transport systems in cell lines is poorly reported.

The genetic component in the inter-individual variability in enzyme activity has been estimated to be high (Wormhoudt et al., 1999). In clinical studies, it is already shown that genetic polymorphisms in biotransformation enzymes and drug transporters can have a large impact on adverse drug reactions and the therapeutic efficacy of drugs like warfarin, several psychoactive drugs and anti-cancer drugs, e.g., irinotecan (Sekine and Saijo, 2001; Scordo et al., 2002; Toffoli et al., 2003). However, in several often used cell line models to study drug metabolism and transport the occurrence of genetic polymorphisms has never been studied, despite the fact that these polymorphisms could influence the outcome of preclinical biotransformation and transport studies of drugs performed with these cell lines. The aim of this study was therefore to screen four often used transformed cell lines in biotransformation and drug transport studies, HepG2, IGROV-1, CaCo-2 and LS180 cells, for polymorphisms in the main CYPs, phase II enzymes and drug transporters.

Materials and methods

Materials. RPMI-1640 medium (with L-glutamine and 25 mM HEPES), heat-inactivated fetal calf serum, penicillin/streptomycin and Hanks' balanced salt solution (pH 7.4) were all obtained from Gibco BRL (Breda, The Netherlands). Primers and DNAzol were purchased from Invitrogen Life Technologies (Paisley, UK) or Metabion (Planegg-Martinsried, Germany) and the restriction enzymes *Bcg*I, *Bsa*I, *BstE*II, *Bst*UI, *Dde*I, *Nci*I, *Nla*III, *Tsp*509 I and *Xcm*I were provided by New England BioLabs (Hitchin, UK). The other restriction enzymes, Taq polymerase and dNTPs were obtained from Fermentas (St. Leon-Rot, Germany). PCR buffers and adjuvants were provided by Stratagene (Optiprime buffer 1-12) (Cedar Creek, TX, USA), Fermentas (*Taq*-buffer) (St. Leon-Rot, Germany) or Applied Biosystems (PCR-buffer II) (Foster City, CA, USA). ExoSAP-IT was obtained from Amersham Biosciences (Uppsala, Sweden) and the Big Dye Terminator Cycle Sequencing Ready Reaction mix v3.1, and AmpliTaq Gold were purchased from Applied Biosystems (Foster City, Ca, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA) and were of analytical grade.

Cell culture growth. The human hepatic carcinoma cell line (Hep G2) and the colon adenocarcinoma cell lines CaCo-2 and LS180 were obtained from the ATCC (Manassas, VA, USA). The human ovarian adenocarcinoma cell line (IGROV-1) was kindly donated by the Netherlands Cancer Institute (Amsterdam, The Netherlands). Routine cultivation of the monolayer cells was performed in RPMI-1640 medium (with L-glutamine and 25 mM HEPES) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were sub-cultured weekly (ratio of 1:5 (v/v) for HepG2, CaCo-2 and LS180 and 1:25 (v/v) for IGROV-1 cells and medium was refreshed after 3 days.

DNA isolation. Genomic DNA was isolated using DNAzol® Reagent according to the instructions of the manufacturer (Invitrogen Life Technologies, Paisly, UK).

PCR amplification. All PCR reactions were performed in a total volume of 25 μl with ~100 ng of genomic DNA, 200 μM dNTPs, 1× PCR buffer, 0.5 U Taq polymerase and 200 nM of forward and reverse primer. To improve the specificity of the PCR adjuvant was added if necessary. AmpliTaq Gold (0.625 U in 25 μl) was used for the PCR sequence reactions. Amplification was carried out on a Biometra T-gradient Thermocycler 96 (Whatman Biometra, Goettingen, Germany). The results of the PCR reactions were analyzed by gel electrophoresis. All PCR methods were validated with sequencing.

β-Globulin was used as internal control in allele-specific PCR (CYP2A6*9) or in case of a deletion of the gene (GSTM1 and GSTT1). The following primers were used to generate a 268-bp product: 5′-CAA CTT CAT CCA CGT TCA CC-3′ (forward) and 5′-GAA GAG CCA AGG ACA GGT AC-3′ (reverse).

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