Drug Metabolism and Pharmacokinetics 30 (2015) 21–29

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Review

Prediction of hepatic and intestinal glucuronidation using *in vitro–in vivo* extrapolation

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
1 October 2014
Available online 13 October 2014

Keywords:
UDP-glucuronosyltransferase
Glucuronidation
In vitro–in vivo extrapolation
Liver
Intestine
Availability
Clearance
Prediction

ABSTRACT

The accurate prediction of hepatic (F_h) and intestinal availability (F_g) is vital for determining human pharmacokinetics. To predict these PK parameters for cytochrome P450 (P450) metabolism, *in vitro–in vivo* extrapolation (IVIVE) using hepatic microsomes, hepatocytes, and intestinal microsomes has been actively investigated. However, IVIVE has not been sufficiently evaluated for non-P450 enzymes. UDP-glucuronosyltransferase (UGT) is a non-P450 enzyme that catalyzes glucuronidation, a major pathway for drugs possessing carboxylic acid, hydroxyl, and amine moieties. In drug metabolism, UGT is the most important enzyme after P450, and prediction of F_h for UGT substrates has mainly been attempted using hepatic models based on the clearance concepts. While various approaches for achieving improved prediction of clearance have been investigated—such as the addition of bovine serum albumin to microsomal incubation mixtures—optimized *in vitro* methods that utilize both hepatic microsomes and hepatocytes for more accurate prediction are still required. Although application of the simplified intestinal availability (SIA) model is effective in predicting the F_g of UGT substrates, this model is limited to compounds with high oral absorption. In this review, we discuss the current state, issues, and future directions of predicting F_h and F_g for glucuronidation.

1. Introduction

Pharmacokinetic factors of attrition have recently been dramatically reduced in drug development [1], due to attempts by pharmaceutical companies to predict human pharmacokinetic profiles of candidate compounds and select only those with appropriate profiles. The accurate prediction of hepatic availability (F_h) is of key importance for that of human pharmacokinetics. Attempts to predict F_h using human biological samples, such as human hepatic microsomes and hepatocytes, have been made via the *in vitro–in vivo* extrapolation (IVIVE) method, based on the clearance concepts [2]. In addition, given the impact of intestinal metabolism on the first-pass effect [3,4], the prediction of intestinal availability (F_g) has also attracted attention. However, IVIVE for predicting F_h has been primarily conducted using compounds metabolized by cytochrome P450 (P450), which is the most important enzyme in drug metabolism [5], with relatively few studies attempting to predict F_h for non-P450 enzymes, such as UDP-glucuronosyltransferase, carbonyl/aldo-keto reductase, aldehyde oxidase, flavin-containing monooxygenase, and monoamine oxidase [6]. Recently, non-P450 enzymes were reported to be involved in human intestinal first-pass metabolism (for example, metabolism of raloxifene by UDP-glucuronosyltransferase [7]). The prediction of human F_g for non-P-450 enzymes is therefore important. However, methods for the prediction of F_g for non-P450 enzymes are also insufficiently evaluated at present. Recently it was reported that IVIVE is useful to predict F_g for P450 [8]. IVIVE may also be useful for predicting F_g of non-P450-metabolized compounds. After P450, UDP-glucuronosyltransferase (UGT) is the most important enzyme in drug metabolism. Indeed, in 2002, UGT was reported to contribute to the clearance of approximately 1 in 10 of the top 200 drugs prescribed in the United States [9].

A comprehensive understanding of methods for predicting hepatic and intestinal glucuronidation catalyzed by UGT may facilitate future drug discovery. There have been several comprehensive reviews regarding the prediction of glucuronidation using IVIVE [10–15]. Here, we review the current methods of predicting F_h and F_g for glucuronidation using IVIVE and discuss our reports and viewpoints.

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http://dx.doi.org/10.1016/j.dmpk.2014.10.001

2. Glucuronidation and UGT

Glucuronidation is catalyzed by UGT in a conjugation reaction between glucuronic acid derived from the cofactor uridine 5′-diphosphate-glucuronic acid (UDPGA) and various xenobiotics, including drugs, dietary chemicals, carcinogens and their metabolites, and endogenous compounds such as bilirubin, fatty acids, and hydroxysteroids [14]. Glucuronidation is a major pathway for drugs that contain carboxylic acid, hydroxyl, and amine moieties [10].

Localized in the endoplasmic reticulum lumen [16], UGT is widely expressed in various tissues, including the liver, kidney, lung, and intestine [17]. Human UGT constitutes four families: UGT1, UGT2, UGT3, and UGT8 [18]. The UGT1A and 2B subfamilies mainly participate in drug metabolism [15]. Ohno and Nakajin [19] reported the expression of 12 UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B15, and UGT2B17) in the human liver and 11 UGT isoforms (UGT1A1, UGT1A3, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17) in the human intestine for UGT1A and 2B subfamilies based on mRNA expression data. Recently, protein quantification methods using liquid chromatography with tandem mass spectrometry (LC-MS/MS) have been applied to the investigation of UGT isoforms. Sato et al. [20] detected the protein expression of 10 UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B15, and UGT2B17) in human hepatic microsomes using an LC-MS/MS-based protein quantification method, which is consistent with a previous report [21]. Sato et al. [20] also detected 6 UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A10, UGT2B7, and UGT2B17) in human intestinal microsomes. Harbourt et al. [22] reported the expression of 7 UGT isoforms (UGT1A1, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10) for UGT1A subfamily in human intestinal microsomes.

and that the substrate concentration used is lower than K_m for enzyme reactions. The advantages of this approach are as follows: it is simple to conduct; it can be applied to many compounds; metabolites do not need to be known; it does not require reference standards of metabolites for measurement (or radiolabels); and it yields $CL_{int,\ in\ vitro}$ based on the disappearance of parent compounds. However, disadvantages of the approach include difficulty in measuring very low $CL_{int,\ in\ vitro}$ values, inability to obtain information on individual metabolites, and inability to obtain K_m and V_{max} parameters [24]. To counter this first disadvantage, a relay method using cryopreserved human hepatocytes was developed [25]. This involves transferring the supernatant from hepatocyte incubations to freshly thawed hepatocytes after a 4 h incubation to prolong exposure to active enzymes in hepatocytes and subsequently measure substrate depletion time profiles.

In order to predict whole body clearance, $CL_{int,\ in\ vitro}$, originally expressed per milligram of microsomal protein or per million cells calculated from *in vitro* metabolism experiments, is expressed per kilogram of body weight through conversion using physiological parameters (microsomal protein content/g of liver or number of hepatocytes/g of liver, and liver weight in g/kg of body weight). Using the scaled $CL_{int,\ in\ vitro}$, hepatic clearance (CL_h) is then predicted using mathematical hepatic models based on the clearance concepts using the blood unbound fraction, f_u, for each drug and the hepatic blood flow, Q_h. The three most widely-used hepatic models are the well-stirred model (equation (1)) [26], the parallel-tube model (equation (2)) [27] and the dispersion model (equation (3)) [28]. The $CL_{int,\ in\ vitro}$ is substituted for the hepatic intrinsic clearance (CL_{int}) in the equations.

$$CL_h = \frac{Q_h \cdot f_u \cdot CL_{int}}{Q_h + f_u \cdot CL_{int}} \quad (1)$$

$$CL_h = Q_h \left[1 - \exp\left(-\frac{f_u \cdot CL_{int}}{Q_h}\right)\right] \quad (2)$$

$$CL_h = Q_h \left[1 - \frac{4a}{(1+a)^2 \exp\left[(a-1)/2D_n\right] - (1-a)^2 \exp\left[-(a+1)/2D_n\right]}\right] \quad (3)$$

3. Prediction of hepatic availability

3.1. Predicting F_h via IVIVE

In this section, we describe the strategies for predicting F_h via IVIVE. *In vitro* intrinsic clearance ($CL_{int,\ in\ vitro}$) is first evaluated from *in vitro* metabolic studies. $CL_{int,\ in\ vitro}$ for glucuronidation is estimated via one of two potential approaches using hepatic microsomes or hepatocytes including UGT.

The first approach involves measuring the production of metabolites over a wide range of compound concentrations and estimated K_m and V_{max}; $CL_{int,\ in\ vitro}$ is then obtained as V_{max}/K_m. With atypical enzyme kinetics, $CL_{int,\ in\ vitro}$ is substituted by CL_{max}, which is the maximum clearance when the enzyme is fully activated [23]. Although this approach has been used as a conventional method, it is difficult to apply to drug discovery when metabolite profiles are not sufficiently characterized and the reference standards of metabolites, which are analytes, are unavailable.

The second approach involves estimating the substrate disappearance rate at a single compound concentration. This approach assumes that substrate disappearance follows a first-order reaction

where D_n (the dispersion number) = 0.17, $a = \sqrt{1 + 4R_nD_n}$ and $R_n = f_u \cdot CL_{int}/Q_h$

Finally, F_h is predicted as follows:

$$F_h = 1 - \frac{CL_h}{Q_h} \quad (4)$$

3.2. *In vitro–in vivo* correlations in experimental animals

Evaluation of *in vitro–in vivo* correlation in experimental animals is important for the development of methods of predicting human pharmacokinetics. Bouska et al. [29] reported that the glucuronidation rate of a series of 5-lipoxygenase inhibitors in cynomolgus monkey microsomes semi-quantitatively correlated with their *in vivo* plasma clearance, which might enable the rank order of compounds in terms of *in vivo* metabolic stability. Mistry and Houston [30] observed that hepatic microsomal $CL_{int,\ in\ vitro}$ values for the glucuronidation of three opioids (morphine, naloxone, and buprenorphine) were consistently lower