Drug Metabolism and Pharmacokinetics 30 (2015) 30–51

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

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

Review

A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development

Shingo Oda [a, 1], Tatsuki Fukami [b, 1], Tsuyoshi Yokoi [a], Miki Nakajima [b, *]

[a] Department of Drug Safety Sciences, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[b] Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
24 November 2014
Accepted 2 December 2014
Available online 11 December 2014

Keywords:
Carboxylesterase
Drug metabolism
Drug development
Enzyme induction
Enzyme inhibitors
Gene expression
Substrate
UDP-glucuronosyltransferase

ABSTRACT

UDP-glucuronosyltransferase (UGT) and esterases are recognized as the most important non-P450 enzymes because of their high contribution to drug metabolism. UGTs catalyze the transfer of glucuronic acid to hydroxyl, carboxyl, or amine groups of compounds, whereas esterases hydrolyze compounds that contain ester, amide, and thioester bonds. These enzymes, in most cases, convert hydrophobic compounds to water-soluble metabolites to facilitate the elimination of compounds from the body. Information about these enzymes is steadily increasing, although our knowledge is still behind our understanding of P450. This review gives an overview of recent findings in UGT and esterases studies focusing on tissue distribution, gene regulation, substrate and inhibitor specificity, and species differences. In particular, the absolute protein content of UGT isoforms and esterases in human tissues could be available. In the field of esterases, it is becoming clear that enzymes other than carboxylesterase are involved in drug hydrolysis. In addition, there is an interesting interplay between UGTs and esterases in the formation and hydrolytic deglucuronidation of acyl-glucuronide, which is considered to be a reactive metabolite. With the growing awareness of the importance of non-P450 enzymes in drug development, issues that should be resolved are discussed.

1. Introduction

Most drugs undergo metabolic biotransformation to form metabolites that are more polar than the parental compounds. Drug metabolism is divided into phase I and phase II reactions. In phase I reactions, polar groups are introduced to the molecules through oxidation, reduction, and hydrolysis. In phase II reactions, phase I metabolites or the parental compounds themselves undergo conjugation reactions with hydrophilic moieties including glucuronic acid, sulfate, glutathione, or amino acids. These reactions increase the water solubility and molecular weight of the compounds and mostly inactivate their biological activities and facilitate their efflux from the cells by transporter, followed by excretion into bile or urine. For clinically used drugs, cytochrome P450 (P450) enzymes contribute to the metabolism of two-thirds of pharmaceutical drugs, with most of the remaining drugs being metabolized by UDP-glucuronosyltransferase (UGT), esterases including carboxylesterase (CES), arylacetamide deacetylase (AADAC), butyrylcholinesterase (BCHE), and paraoxonase (PON), and flavin-containing monooxygenase (FMO) [1].

Developing a compound with desirable absorption, distribution, metabolism, and excretion (ADME) properties is essential to selecting candidate compounds in drug development. In 1991, inappropriate pharmacokinetics and bioavailability were the leading causes of attrition of a drug from the market and accounted for approximately 40% of all attrition. By 2000, these factors had been dramatically reduced to less than 10% of attrition [2,3], probably owing to accumulated information on ADME, especially for P450 enzymes. Currently, there is a trend in drug development strategy to reduce the lipophilicity of new chemical entities, which has consequently made the non-P450 enzymes more prominent contributors to the clearance of drug candidates. Therefore, a better understanding of non-450 enzymes is required for drug development.

In this review article, we focus on UGT and esterases because these enzymes are high contributors to drug metabolism. Although information regarding these enzymes is behind that for the P450 enzymes, understanding of these enzymes is steadily increasing at

* Corresponding author.
E-mail address: nmiki@p.kanazawa-u.ac.jp (M. Nakajima).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dmpk.2014.12.001

a molecular and functional level. This review attempts to summarize recent progress and the present knowledge of UGTs and esterases in terms of their tissue distribution, gene regulation, substrates and inhibitors, and species differences that are useful in drug development and academic research.

2. UDP-glucuronosyltransferase

2.1. Overview of UDP-glucuronosyltransferase

UGTs (EC 2.4.1.17) are a family of conjugating enzymes that play important roles in the metabolism of endogenous and exogenous compounds [4]. UGTs catalyze the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to hydroxyl, carboxyl, or amine groups of hydrophobic compounds [5]. Mammalian UGTs are divided based on evolutionary divergence into two families, UGT1 and UGT2, that can be further divided into three subfamilies, UGT1A, UGT2A, and UGT2B [4]. The human *UGT1A* gene cluster is located on chromosome 2q37 and contains multiple unique first exons, as well as the conserved exons 2–5, which can give rise to nine types of functional UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10. *UGT1A2, UGT1A11, UGT1A12,* and *UGT1A13* are pseudogenes and do not produce functional proteins. The *UGT2A* and *UGT2B* genes are located on chromosome 4q13 and encode three and seven functional proteins, respectively. UGT2A1 and UGT2A2 are formed by the alternative splicing of variable first exons and common exons 2–6, likely the *UGT1A* gene, whereas UGT2A3 and each UGT2B isoform including UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28 are encoded by individual genes. UGT proteins consisting of approximately 530 amino acids are localized on the luminal side of the endoplasmic reticulum (ER) with a C-terminal transmembrane domain. The C-terminal half of UGT proteins is highly conserved across isoforms and is thought to be involved in UDPGA binding, whereas the N-terminal half is divergent and is regarded as a substrate binding domain that confers overlapping but distinct substrate and inhibitor selectivities to UGTs.

2.2. Tissue distribution of human UGTs

Determining the UGT distribution in human tissues has become an interesting research question. Because UGTs share high nucleotide or amino acid sequence similarity, the preparation of isoform-specific antibodies is not easy. Despite the high degree of amino acid sequence similarity among UGTs, some studies successfully developed specific antibodies against individual UGT isoforms such as UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9, and evaluated UGT tissue distribution by western blotting [6–8]. However, because of the limited availability of specific antibodies, comprehensive analyses of UGT protein expression had been a task to be accomplished. On the contrary, designing primers or oligonucleotides that can specifically detect each UGT isoform is relatively easy. Hence, earlier studies on the tissue distribution of individual human UGT isoforms were performed using northern blotting or reverse transcription-PCR (RT-PCR) analysis [9–11]. Although these methods successfully detected individual UGT isoforms, the results obtained were not quantitative and often lacked specificity for each isoform.

Quantitative RT-PCR (qRT-PCR) analysis enables the measurement of the absolute content of individual UGTs in various tissues [12–14]. qRT-PCR analysis also allows the comparison of UGT content across isoforms in a given tissue. The results of three studies were summarized by Rowland et al. (2013) [15]. Accordingly, members of the UGT2B subfamily account for almost 75% of the total hepatic UGT expression. The expression of individual UGTs at the mRNA level in the liver are as follows: UGT2B4 (45%) > UGT2B15 (13%) > UGT2B10 (11%) > UGT2B7 (7%) > UGT1A1 (7%) > UGT1A9 (6%) > UGT1A4 (5%) > UGT1A6 (5%) > UGT2B17 (1.5%) > UGT1A3 (1.3%) [15]. No or negligible hepatic expression was reported for UGT1A5, UGT1A7, UGT1A8, UGT1A10, UGT2A1, and UGT2A2.

Total UGT mRNA expression in the small intestine seems to be one-seventh of that in adult liver [14]. When combining the results of from Ohno and Nakajin (2009) and Court et al. (2012), UGT2B7 (20%) > UGT2B17 (18.7%) > UGT1A10 (17.3%) > UGT1A1 (12.6%) > UGT1A6 (8.7%) account for approximately 80% of total UGT mRNA expression in the small intestine [15]. Among the different UGT isoforms, UGT1A10 is specifically expressed in intestines, and UGT1A8, which shares 97% amino acid similarity with UGT1A10, is also specifically expressed in the intestine, although its expression is approximately 22-fold lower than that of UGT1A10 [13,14]. UGT1A4, UGT2B4, and UGT2B10, which are abundant in the liver, are consistently absent in the small intestine, and the results from the two studies contradict one another with regard to UGT2B17 expression [13,14]. A large interindividual variability in the mRNA expression levels of UGT in the liver and small intestine should also be noted.

In tissues other than the liver and intestines, Court et al. (2012) reported that UGT1A6 (46%) > UGT2B17 (13%) > UGT2A1 (12%) > UGT1A10 (9%) > UGT2A2 (5%) are expressed at mRNA level in the nasal mucosa, and UGT2B7 (37%) and UGT1A9 (36%) are expressed in the kidney [14], supporting previous northern blot and RT-PCR data [14]. Although a previous study found that UGT1A7 is expressed in the stomach [10], Court et al. (2012) reported that it is expressed at a negligible level in the stomach but is relatively highly expressed in the esophagus [14].

Recent progress in LC-MS or LC-MS/MS technologies has enabled investigators to quantify the protein content of individual UGTs [16–23]. The evaluated UGT protein contents in the liver, small intestine, kidney, and recombinant UGT expression systems are summarized in Tables 1 and 2. The members of the UGT2B subfamily account for 54% of the hepatic total UGT protein content. UGT2B7 showed the highest protein content (98 pmol/mg, 22% of total hepatic UGT content). UGT2B4 accounts for 13% of total protein content, although it is abundantly (45%) expressed at the mRNA level. UGT2B15 and UGT1A4 each account for 12% of the total UGT protein content, followed by UGT1A1 (10% of total), UGT1A6 (10% of total), UGT1A3 (7% of total), UGT1A9 (7% of total), UGT2B10 (4% of total) and UGT2B17 (4% of total). Although the mean value was not indicated, UGT2B17 protein content showed a large interindividual variability (3146-fold). The protein was not detected in 18 of 60 individual HLMs [20], which is likely due to the *UGT2B17* defective allele, *UGT2B17*2* [24]. The other hepatic UGTs showed 2.5- to 25.2-fold interindividual variability.

Absolute UGT protein contents in the small intestine were reported by two studies (Table 2) [16,22]. Harbourt et al. (2012) reported that most of the UGT1A isoforms including UGT1A1 (7.2 pmol/mg), UGT1A4 (5.3 pmol/mg), UGT1A6 (2.3 pmol/mg), UGT1A7 (8.4 pmol/mg), UGT1A8 (6.1 pmol/mg), UGT1A9 (6.6 pmol/mg), and UGT1A10 (4.7 pmol/mg) [16] are expressed in the small intestine. Sato et al. (2014) reported that the UGT2B17 content is the highest (112.0 pmol/mg), followed by UGT1A1 (39.6 pmol/mg), UGT1A10 (17.9 pmol/mg), UGT2B7 (15.7 pmol/mg), UGT1A3 (1.9 pmol/mg), and UGT1A4 (1.6 pmol/mg) [22]. UGT2B17 is known to metabolize C_{19} steroids such as androsterone, dihydrotestosterone, and testosterone; however, the physiological significance of UGT2B17 in the small intestine remains to be clarified. The absolute UGT protein contents in the kidney were also reported by three studies [16,19,22]. Sato et al. (2014) reported the protein content of