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Perspective

Advances in drug metabolism and pharmacogenetics research in Australia

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ABSTRACT

Metabolism facilitates the elimination, detoxification and excretion in urine or bile (as biotransformation products) of a myriad of structurally diverse drugs and other chemicals. The metabolism of drugs, non-drug xenobiotics and many endogenous compounds is catalyzed by families of drug metabolizing enzymes (DMEs). These include the hemoprotein-containing cytochromes P450, which function predominantly as monooxygenases, and conjugation enzymes that transfer a sugar, sulfate, acetate or glutathione moiety to substrates containing a suitable acceptor functional group. Drug and chemical metabolism, especially the enzymes that catalyse these reactions, has been the research focus of several groups in Australia for over four decades. In this review, we highlight the role of recent and current drug metabolism research in Australia, including elucidation of the structure and function of enzymes from the various DME families, factors that modulate enzyme activity in humans (e.g. drug-drug interactions, gene expression and genetic polymorphism) and the application of in vitro approaches for the prediction of drug metabolism parameters in humans, along with the broader pharmacological/clinical pharmacological and toxicological significance of drug metabolism and DMEs and their relevance to drug discovery and development, and to clinical practice.

1. Introduction

Maintenance of chemical homeostasis and the elimination of low molecular weight compounds are mediated by families of enzymes, generally referred to as drug metabolising enzymes (DMEs), found predominantly in the liver, kidneys and gastrointestinal tract. DMEs typically catalyse the formation of more water-soluble metabolites that are more readily removed from cells and excreted in the urine and/or bile [1], thereby limiting the excessive accumulation of chemicals in membranes and other lipophilic compartments and reducing their capacity for harm. Many of the substrates of DMEs are from sources outside the body (xenobiotics), including environmental pollutants and toxins, and non-nutrient chemicals in food such as plant bioflavones. As implied by the name "DME", drugs comprise an important group of substrates. Indeed, more than 70% of all clinical drugs are eliminated primarily by metabolism. Thus, apart from facilitating detoxification and elimination in urine and bile (as polar metabolites), metabolism represents a clearance mechanism for the majority of drugs. As such, the efficiency of metabolism is a determinant of drug dose, since drug dose rate is given by the product of systemic clearance and the 'target' concentration of the drug in blood. Other compounds that are processed by DMEs originate from endogenous metabolism. These include steroids, bile acids, biogenic amines, neurotransmitters, fatty acid metabolites, fat-soluble vitamins and products of heme breakdown [2]. Hence, the action of DMEs is important in modulating ligands involved in pathways regulating cell function and survival.

DME families include cytochrome P450, UDP glycosyltransferase, sulfotransferase, *N*-acetyltransferase, and glutathione-S-transferase. Research in Australia has contributed significantly to the current understanding of the structure, function and regulation of many DMEs along with their importance in pharmacology and therapeutics, toxicology, and physiology.

Abbreviations: BSA, bovine serum albumin; CYP, cytochrome P450; DDI, drug-drug interaction; DME, drug metabolizing enzyme; GST, glutathione-*S*-transferase; HLM, human liver microsomes; HSA, human serum albumin; NAT, *N*-acetyltransferase; NSAID, non-steroidal anti-inflammatory agent; SULT, sulfotransferase; UGT, UDP-glycosyltransferase.

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2. UDP glycosyltransferases

The UDP glycosyltransferases (UGT) have been an important focus of the Drug Metabolism Group at Flinders University for more than 30 years. UGTs are localised in the endoplasmic reticulum in cells of various organs and tissues, predominantly the liver, gastrointestinal tract and kidneys [3]. This location in a bilayer membrane is ideally suited for access to lipophilic substrates and the subsequent transfer of the sugar moiety from UDP sugars (the co-substrate) to the substrate (aglycone) to form ß-D-glycosides. The attachment of a sugar, in general, reduces the biological effects and toxicity of the lipophilic chemical and increases its water solubility to facilitate excretion in the bile and/or urine [1]. Each of the 22 functional UGTs in the human genome glycosidate a variety of different compounds and, like the cytochromes P450, exhibit distinct but sometimes overlapping substrate selectivities. Hence, UGTs together facilitate the elimination of a myriad of structurally diverse aglycones that include drugs, non-drug xenobiotics (e.g. dietary chemicals, environmental pollutants) and endobiotics (e.g. bilirubin, eicosanoids, fatty acids, steroid hormones) [3,4]. As UGTs are orientated in the endoplasmic reticulum with their catalytic sites on the luminal side of the membrane bilayer, transporters are required to transfer UDP-sugars from their site of synthesis in the cytoplasm to the lumen of the endoplasmic reticulum. It has been demonstrated that human liver microsomal UDP-glucuronic acid uptake, which is trans-stimulated by alternate UDP-sugars, exhibits biphasic kinetics consistent with the involvement of at least two proteins in cofactor transport [5]. Further, UDP-glucuronic acid transport was shown to be inhibited by several drugs, particularly nucleoside analogues, suggesting the possibility of impaired glucuronidation due to reduced cofactor availability.

Glucuronidation, which is catalyzed by members of the UGT1 and UGT2 families, is the dominant metabolic route for most drugs, non-drug xenobiotics and endogenous compounds that contain an acceptor functional group (especially hydroxyl- (aliphatic or phenolic), carboxylate and amine) suitable for conjugation with glucuronic acid [1]. Further, UGT1 and UGT2 family enzymes are involved in a range of clinically important drug-drug interactions, and variation in UGT function and/or expression contributes to interindividual differences in drug disposition as well as to cancer risk [6–8].

The human UGT1 protein family contains nine functional members with different amino-terminal domains (encoded by exons A1, A3, A4, A5, A6, A7, A8, A9 and A10) and an identical carboxyl-terminal domain (encoded by a shared set of 4 downstream exons 2–5) [4]. Each UGT1 transcript is generated from a unique first exon (A1 or A2, etc) with its associated promoter and exons 2–5. The human UGT2 family is subdivided into two subfamilies; the UGT2A family containing three members (UGT2A1, UGT2A2 and UGT2A3) and the UGT2 B family containing seven members (UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28) [4]. The two UGT families are differentially expressed in the liver and a range of other tissues including the gastrointestinal tract, kidney, olfactory epithelium, ovary, lung, breast, testis and prostate [3]. They also display marked interindividual variation in levels within a tissue [7]. Hepatically expressed enzymes of the UGT 1A and 2B subfamilies appear to be of greatest significance to drug clearance in humans, especially UGT 1A1, 1A4, 1A6, 1A9, 2B7 and 2B15 [6].

Although glucuronides are the main sugar conjugates formed in humans, conjugates with glucose, galactose, xylose and *N*-acetylglucosamine are also detected [2]. These latter conjugates are usually minor components of the total pool of conjugates in blood and urine. For example, although the main metabolites of morphine are the 3 and 6-glucuronides, glucosides of morphine have been detected [9]. The formation of morphine glucosides is catalyzed by UGT2B7, which is also the primary enzyme responsible for the glucuronidation of this opioid [10]. Glucuronidation is the dominant metabolic pathway for morphine because the binding affinity of UDP-glucuronic acid to UGT2B7 is higher than that of UDP-glucose [9]. The ability of UGT2B7 and a number of other enzymes to utilize both UDP-glucuronic acid and UDP-glucose as cofactor demonstrates that glucuronidation and glucosidation may occur as complementary, parallel metabolic pathways for numerous aglycones.

Occasionally, however, the major metabolite is not the glucuronide but a conjugate with other sugars that are not commonly utilized by UGT1 and UGT2 enzymes (e.g. galactose, *N*-acetylglucosamine) [11]. For example, although ursodeoxycholic acid is normally a low abundance secondary bile acid in human urine, when given as a drug for cholestatic liver disease and to dissolve gallstones, it can constitute up to 50% of total bile acids in blood, with the *N*-acetylglucosamine conjugate as the major metabolite [12]. Based on such observations, a search for enzymes involved in conjugating lipophilic compounds with sugars other than glucuronic acid was initiated, resulting in the discovery of the UGT3 family and a previously unrecognized activity of the ceramide galactosidating enzyme, UGT8 [11,13,14].

The UGT3 family contains two members, UGT3A1 and UGT3A2 whereas the UGT8 family contains only one member, UGT8. In contrast to the UGT1 and UGT2 families, these three UGTs do not use UDP-glucuronic acid as a cofactor. UGT3A1 and UGT3A2 were shown to utilise UDP-*N*-acetylglucosamine [11] and UDP-glucose and UDP-xylose, respectively [13]. UGT8 uses UDP-galactose almost exclusively [14]. The UGT3 enzymes conjugate a range of drugs, non-drug xenobiotics and endobiotics and are highly expressed in common sites of detoxification such as kidney, liver, and intestine, as well as lower levels in brain, lung, skin, and testis. UGT8 is highly expressed in the brain, intestine and kidney where it conjugates galactose to ceramides, bile acids and bile acid-like drugs [14].

2.1. UGT structure-function

2.1.1. Determinants of UDP-sugar selectivity

The discovery that some UGTs use sugars other than glucuronic acid in the conjugation of drugs and drug-like chemicals prompted an investigation of the structural features of UGT enzymes that determine UDP-sugar selectivity. Four critical amino acids at the terminus of the C-terminal domain signature sequence of UGTs that alter UDP-sugar preference were identified [15]. An asparagine (Asn-391) in the UGT signature sequence of UGT3A1 is necessary for utilization of UDP-*N*-acetylglucosamine, whereas a phenylalanine favors UDP-glucose use in UGT3A2. An analysis of homology models docked with UDP-sugar donors indicates that Asn-391 in UGT3A1 is able to accommodate the *N*-acetyl group on C2 of UDP-*N*-acetylglucosamine so that the anomeric carbon atom (C1) is optimally situated near to His-35, the amino acid that is considered to act as the catalytic base in glucuronidation reactions [16]. The capacity of UGT8 to use UDP-galactose was dependent on residue His-383 at the carboxyl-terminal end of the signature sequence [14].

2.1.2. Determinants of aglycone selectivity

The molecular basis of UGT enzyme aglycone and inhibitor selectivities has been investigated using a number of approaches. An *N*-terminal domain His, which is believed to function as the catalytic base in the *O*-glucuronidation of hydroxy-containing aglycones, is conserved in all UGT1A and UGT2B subfamily enzymes except UGT1A4 (Pro-40) and UGT2B10 (Leu-34). Unlike other UGT enzymes, UGT1A4 and UGT2B10 lack the ability to glucuronidate planar phenols. Rather, these two enzymes preferentially cat-