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Review

Toxicological potential of acyl glucuronides and its assessment

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ABSTRACT

Idiosyncratic drug toxicity (IDT) is a serious problem in drug development. Reactive metabolites are postulated to be one of the causes for IDT. Conjugated metabolites are generally non-reactive except for acyl glucuronides (AGs), which are sufficiently reactive to covalently bind to endogenous proteins. Thus, it has been suggested that AGs would contribute to IDT caused by carboxylic acid-containing drugs. Glucuronidation of a carboxylate residue is catalyzed by UDP-glucuronosyltransferase 1A and 2B isoforms. Unstable AGs undergo intramolecular rearrangements as well as non-enzymatic and enzymatic hydrolysis. The instability and reactivity toward proteins have been well studied for a large number of AGs. Moreover, the half-life of AGs in neutral buffer is becoming a common marker for the prediction of toxicity caused by carboxylic acid-containing drugs in the screening of new chemical entities; however, the underlying mechanisms of the toxicity are not elucidated. Recently, an immunostimulation assay has been proposed for the assessment of the toxicological potential of AGs, which may have a better predictability compared with half-life and peptide adduct assays. In addition to *in vitro* studies, studies in model animals indicate the *in vivo* toxicological potential of AGs and help understand the mechanisms of the AG toxicity.

1. Introduction

Idiosyncratic drug toxicity (IDT) is one of the great concerns in drug discovery because it has forced some drugs to be withdrawn from the market. IDT has been postulated to be caused by reactive metabolites that covalently bind to endogenous macromolecules [1]. Reactive metabolites include electrophilic structures, such as quinone, epoxide, and aldehyde, generated by oxidative biotransformation. As a typical example, *N*-acetyl-*p*-benzoquinone imine, an oxidative reactive metabolite of acetaminophen, shows various toxic effects, such as cytotoxicity, oxidative stress, depletion of glutathione (GSH), and immune activation [2]. In addition to oxidative reactive metabolites, acyl glucuronides (AGs), which are generated by UDP-glucuronosyltransferase (UGT) via conjugation with glucuronic acid, have also been suspected to contribute to IDT because of their instability, although conjugation is generally considered a detoxification pathway. This view is supported by the fact that 17 of 121 drugs withdrawn worldwide between 1960 and 1999 were carboxylic acid-containing drugs [3].

Importantly, the Metabolites in Safety Testing (MIST) guidance published by the Food and Drug Administration states that AGs are considered toxic metabolites, and safety assessment may be needed [4]. Characterization of AGs has been performed since more than half a century ago, but the toxicological potential of AGs still remains unclear. Initially, the physicochemical aspects, such as stability and reactivity, were studied [5]. Then, biological and immunological properties of AGs were uncovered to gain better understanding of their relevance to the toxicity caused by carboxylic acid-containing drugs.

In particular, the uncertainty regarding the *in vivo* toxicological potential of AGs is one of the reasons why the contribution of AGs to IDT is still obscure. However, the *in vivo* toxicological potential

Abbreviations: ADR, adverse drug reaction; AG, acyl glucuronide; AGE, advanced glycation end product; APC, antigen-presenting cell; BCRP, breast cancer resistance protein; DDI, drug–drug interaction; GSH, glutathione; HSA, human serum albumin; IDT, idiosyncratic drug toxicity; IL, interleukin; KO, knockout; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MAPK, mitogen-activated protein kinase; MIST, Metabolites in Safety Testing; MRP, multidrug resistance protein; NSAID, non-steroidal anti-inflammatory drug; OATP, organic anion-transporting polypeptide; PBMC, peripheral blood mononuclear cell; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

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has recently been revealed for several AGs using model animals (e.g., inhibition of deglucuronidation, efflux transporter-knockout [KO] animals). Based on the findings from both *in vitro* and *in vivo* studies, risk consideration of AGs would prevent the IDT caused by carboxylic acid-containing drugs. In this review article, we summarize the toxicological potential of AGs and its assessment based on physicochemical and biological effects.

2. Formation and degradation of AGs

2.1. AG formation by UGT

Carboxylic acid-containing drugs are converted to AGs via glucuronidation by various UGT isoforms (Table 1) [6–32]. Nonsteroidal anti-inflammatory drugs (NSAIDs) (diclofenac, flurbiprofen, ibuprofen, ketoprofen, and naproxen), mycophenolic acid, valproic acid and gemfibrozil are mainly glucuronidated by UGT2B7 [10,11,17,32]. In addition to UGT2B7, etodolac, furosemide, probenecid and montelukast are predominantly metabolized to their AGs by UGT1A isoforms, such as UGT1A1, UGT1A3, and UGT1A9 [11,16,25]. Fluoroquinolone antibiotics such as levofloxacin, grepafloxacin, moxifloxacin, and sitafloxacin are not conjugated by UGT2B7 but are conjugated by UGT1A1 and UGT1A9 [18]. In another study, the glucuronidation of trovafloxacin was found to be mainly catalyzed by UGT1A1 [30]. Interestingly, there is a stereoselective difference in the metabolic properties of UGT2B7 and UGT1A9 toward glucuronidation of flurbiprofen enantiomers [33]. In the UGT2B7-catalyzed glucuronidation, the intrinsic clearance of *R*-flurbiprofen is 2.1-fold higher than that of *S*-flurbiprofen, whereas in the UGT1A9-catalyzed glucuronidation, the intrinsic clearance of *R*-flurbiprofen is 1.7-fold lower than that of *S*-flurbiprofen. In contrast to UGT1A1, UGT1A3, UGT1A9, and UGT2B7, carboxylic acid-containing drugs that are predominantly glucuronidated by UGT1A4 or UGT2B15 are not yet known, likely because glucuronidation activities of UGT1A4 and UGT2B15 are lower than those of other UGT isoforms, as shown for NSAIDs, probenecid, and valproic acid [10,11,25,32].

Recently, absolute UGT protein expression levels have been determined in human liver, small intestine, and kidney by liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis. UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, and UGT2B15 were found to be abundant in human liver, while UGT1A6, UGT1A9, and UGT2B7 were shown to be highly expressed in human kidney [34–36]. Considering the intrinsic clearance and protein expression level for each isoform, most carboxylic acid-containing drugs are mainly glucuronidated by UGT1A1, UGT1A3, UGT1A9, or UGT2B7 in human liver. Unlike its low expression in the liver and kidney, the UGT1A10 protein is highly expressed in the

Table 1
List of UGT isoforms metabolizing carboxylic acid-containing drugs.

Drug	Category	UGT isoform												Refs
		1A1	1A3	1A4	1A6	1A7	1A8	1A9	1A10	2B4	2B7	2B15	2B17	
Benoxaprofen[b]	NSAIDs	–	–	–	–	–	–	–	–	–	Y	–	–	[6]
Bumetanide	Loop diuretics	–	–	–	–	–	–	Y	–	–	–	–	–	[7]
Clofibric acid	Lipid lowering drugs	N	Y	–	–	Y	Y	–	Y	–	Y	–	–	[6,8,9]
Diclofenac	NSAIDs	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y[a]	Y	Y	[9–12]
Diflunisal	NSAIDs	N	Y	–	–	Y	Y	Y	Y	–	Y	–	–	[6,7,9,13]
Etodolac	NSAIDs	N	Y	N	Y	Y	Y	Y[a]	Y	–	Y	Y	–	[14]
Fenoprofen	NSAIDs	N	Y	–	–	Y	Y	Y	Y	–	Y	–	–	[6,7,9]
Flufenamic acid	NSAIDs	–	–	–	–	–	–	Y	–	–	Y	–	–	[15]
Flurbiprofen	NSAIDs	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	[9–11]
Furosemide	Loop diuretics	Y	Y	N	Y	Y	Y	Y[a]	Y	N	Y	N	N	[7,9,16]
Gemfibrozil	Lipid lowering drugs	Y	Y	N	Y	N	N	Y	N	Y	Y[a]	N	Y	[10,17]
Grepafloxacin	Antibiotics	Y	Y	N	N	Y	N	Y[a]	N	–	N	–	–	[18]
Ibufenac[b]	NSAIDs	–	–	–	–	–	–	–	–	–	–	–	–	–
Ibuprofen	NSAIDs	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	–	Y	[9–11,19]
Indomethacin	NSAIDs	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y[a]	Y	Y	[9,11,20]
Isoxepac[b]	NSAIDs	–	–	–	–	–	–	–	–	–	–	–	–	–
Ketoprofen	NSAIDs	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	[10,11,21]
Levofloxacin	Antibiotics	Y[a]	Y	N	N	Y	N	Y[a]	N	–	N	–	–	[18]
Meclofenamic acid	NSAIDs	–	–	–	–	–	–	–	–	–	–	–	–	–
Mefenamic acid	NSAIDs	N	Y	–	–	Y	Y	Y	Y	–	Y	–	–	[9,15]
Montelukast	Leukotriene receptor antagonist	Y	Y[a]	N	Y	Y	Y	Y	N	N	N	N	N	[22]
Moxifloxacin	Antibiotics	Y[a]	Y	N	N	Y	N	Y	N	–	N	–	–	[18]
Mycophenolic acid	Immunosuppressive drugs	Y	N	N	N	N	N	N	N	Y	Y[a]	–	–	[23]
Naproxen	NSAIDs	Y	Y	N	Y	N	Y	Y	Y	Y	Y	N	Y	[6–8,11]
Niflumic acid	NSAIDs	Y[a]	Y	N	N	N	Y	Y	Y	–	Y	–	–	[10,24]
Oxaprozin	NSAIDs	–	–	–	–	–	–	–	–	–	–	–	–	–
Probenecid	Uricosuric drugs	Y[a]	Y	Y	N	Y	Y	Y[a]	Y	N	Y[a]	Y	Y	[25]
Repaglinide	Antidiabetics	Y[a]	Y	N	N	Y	Y	Y	N	N	Y	N	Y	[26]
Salicylic acid	NSAIDs	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	N	[9,27]
Sitafloxacin	Antibiotics	Y[a]	Y	N	N	Y	N	Y	N	–	N	–	–	[18]
Sulindac	NSAIDs	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N	[11]
Suprofen	NSAIDs	N	Y	N	N	N	N	Y	N	–	Y	–	–	[10]
Telmisartan	Angiotensin receptor blocker	Y	Y[a]	Y	N	Y	Y	Y	Y	Y	Y	N	N	[28,29]
Tolmetin	NSAIDs	–	–	–	–	–	–	–	–	–	–	–	–	–
Trovafloxacin	Antibiotics	Y[a]	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	[30]
Valproic acid	Antiepileptic drugs	N	Y	Y	Y	N	Y	Y	Y	–	Y[a]	N	–	[10,31,32]
Zomepirac[b]	NSAIDs	–	–	–	–	–	–	–	–	–	Y	–	–	[6]

Y: detected, N: not detected, –: not determined.
NSAID: Non-steroidal anti-inflammatory drug.
[a] Estimated responsible isoforms in liver.
[b] Withdrawn drugs.

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