

Commentary

Safety Assessment of Acyl Glucuronides—A Simplified Paradigm

Dennis A. Smith, Timothy Hammond, and Thomas A. Baillie

4 The Maltings, Walmer, Kent, United Kingdom (D.A.S.); Preclinical Safety Consulting Ltd., Loughborough, Leicestershire, United Kingdom (T.H.); and Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, Washington (T.A.B.)

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ABSTRACT

While simple *O*- (ether-linked) and *N*-glucuronide drug conjugates generally are unreactive and considered benign from a safety perspective, the acyl glucuronides that derive from metabolism of carboxylic acid-containing xenobiotics can exhibit a degree of chemical reactivity that is dependent upon their molecular structure. As a result, concerns have arisen over the safety of acyl glucuronides as a class, several members of which have been implicated in the toxicity of their respective parent drugs. However, direct evidence in support of these claims remains sparse, and due to frequently encountered species differences in the systemic exposure to acyl glucuronides (both of the parent drug and

oxidized derivatives thereof), coupled with their instability in aqueous media and potential to undergo chemical rearrangement (acyl migration), qualification of these conjugates by traditional safety assessment methods can be very challenging. In this Commentary, we discuss alternative (non-acyl glucuronide) mechanisms by which carboxylic acids may cause serious adverse reactions, and propose a novel, practical approach to compare systemic exposure to acyl glucuronide metabolites in humans to that in animal species used in preclinical safety assessment based on relative estimates of the total body burden of these circulating conjugates.

Introduction

Simple *O*- (ether) and *N*-glucuronide conjugates of drugs generally are considered to have a low potential to reversibly interact with proteins and trigger pharmacological or toxicological effects (the conjugation dramatically lowers lipophilicity and increases polar surface area). In this regard, such conjugates usually do not require further studies from a safety assessment standpoint, even when formed in human in an excess amount. Rare exceptions do occur, such as morphine-6-glucuronide which is equiactive as a mu-opioid receptor agonist to the parent drug.

Some caution is applied in the case of acyl glucuronides, whose disposition and possible safety risk have been reviewed extensively (Faed, 1984; Spahn-Langguth and Benet, 1992; Bailey and Dickinson, 2003; Shipkova et al., 2003; Regan et al., 2010). While the reversible interaction of such conjugates with proteins is of equally low frequency to those of ether-linked or *N*-glucuronides, acyl glucuronides have the ability to directly acylate proteins and to undergo intramolecular rearrangements producing reactive aldehydes that lead to protein glycation (Stachulski, 2011; Monrad et al., 2014). Moreover, it is now known that certain acyl glucuronides are responsible for clinically relevant drug-drug interactions. Thus, the lipid-lowering agent gemfibrozil causes time-dependent inhibition of CYP2C8, not by the parent drug, but by its acyl glucuronide conjugate (Shitara et al., 2004; Ogilvie et al., 2006). A similar phenomenon has been described for the carboxylic acid metabolite of the antiplatelet agent clopidogrel (Tornio et al., 2014).

Recent publications on this topic from industry and regulatory authority authors (Luffer-Atlas and Atrakchi, 2017) have included statements such as, "...not all disproportionate human metabolites (i.e., present only in humans or present at higher plasma concentrations in humans than in the animals used in nonclinical studies) must be covered in animal studies. While simple *O*-glucuronides, *O*-sulfates, and quaternary *N*⁺-glucuronides may be considered benign from a human safety perspective, animal coverage of acyl glucuronides could still be warranted because of reactivity concerns." Indeed, the Food and Drug Administration guidance on the topic refers to acyl glucuronides as toxic compounds in what appears to be a broad generalization regarding this entire class of drug metabolites (see <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>) despite evidence for in vivo toxicity attributable to an acyl glucuronide being sparse.

Drugs with carboxylic acid moieties often form acyl glucuronides. These are generated in combination with oxidative metabolites, which as a general rule are subject to oxidation at sites remote in the molecule from the acidic function. These oxidized metabolites may also form acyl glucuronides, and the circulating and excreted metabolites will represent a combination of these processes. Species differences in proportion are likely due to this combination of processes. For instance, the dog is known to be much less capable of oxidative metabolism of a number of acidic drugs than other species. Moreover, the excretion of acyl glucuronides is dependent to some degree on their stability, which in a biologic system depends not just on β -glucuronidases, but also on other hydrolytic enzymes such as esterases. These complexities in the formation and elimination of acyl glucuronides contribute to marked species differences in their systemic exposure, both in the healthy

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ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; SJS, Stevens-Johnson syndrome.

condition and in disease states (Liu and Smith, 2006). For example, rodents are recognized as having much higher esterase activity in their plasma and blood than other species. Thus, during safety evaluation it is likely that humans, who readily oxidize acidic drugs, may have potentially abundant acyl glucuronides of oxidative metabolites of the drug in circulation and excreta. In contrast, in rat the oxidative metabolites, and in dog the acyl glucuronide of the parent, may be the major forms detected.

How acyl glucuronides are best considered in metabolites in safety testing evaluations remains unclear. In that context, general principles are needed to establish what constitutes an unqualified acyl glucuronide metabolite, especially when species differences are likely to arise and there is no compelling universal rationale based on cause and effect that would allow for assessing their role as potential toxic entities. In this Commentary, we propose an approach to this problem in which all acyl glucuronide metabolites formed from a given drug in a particular species are considered collectively, as opposed to individually, in assessing compliance with regulatory guidance aimed at qualification of metabolites.

Discussion

History of Association of Acyl Glucuronides with Toxicity—Adverse Effects of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). That acyl glucuronides can react covalently with proteins through acylation (a potentially reversible process) or glycation (an irreversible reaction) is now well recognized, but the downstream consequences of these phenomena are unknown. Classically, relationships have been drawn between acyl glucuronide formation and a causal role in hypersensitivity or Stevens Johnson-syndrome (SJS) for NSAIDs such as zomepirac, based on the premise that protein reactivity is linked to various diverse toxicities (Smith et al., 1990). Zomepirac was the historical precedent for this view and its glucuronide is an example of the most unstable acyl glucuronides (discussed subsequently), and therefore can be considered to be a worst-case example. The major toxicities that led to the withdrawal of zomepirac are common to many acidic drugs including other NSAIDs. While many carboxylic acid-containing compounds form acyl glucuronides of varying stability, a sizable portion do not, such as acetaminophen, piroxicam, valdecoxib, and celecoxib. Indeed, analysis shows that SJS is most pronounced (but still of low incidence) with oxamic derivatives (<2 cases per million users per week) and celecoxib (six cases per million person per year) compared with the carboxylic acid-containing NSAIDs (Nanau and Neuman, 2010). These toxicities are complex and no single mechanism explains all of the findings. Moreover, there is no definitive evidence that acyl glucuronide metabolites are responsible for these serious adverse effects. Both hypersensitivity and SJS are influenced by the mechanism of action of NSAIDs. Inhibition of cyclooxygenase decreases the production of anti-inflammatory metabolites of arachidonic acid, such as prostaglandin E₂, and in enhanced processing of arachidonic acid by 5- and 15-lipoxygenase enzymes. This, in turn, results in overproduction of leukotrienes and 5- and 15-hydroxyicosatetraenoic acids which have a proinflammatory pharmacology. While the SJS and hypersensitivity reactions may also have an immune basis (with possible involvement of reactive metabolites), the frequency of these reactions does not correlate with the formation of acyl glucuronides or their stability. These reactions are classified as urticaria, angioedema, and anaphylaxis induced by a single NSAID rather than by cross reactivity to multiple compounds. Historically, they are most frequently associated with the now little-used pyrazolones (antipyrine, aminopyrine, and dipyrone, which do not form acyl glucuronides), but are also reported for aspirin, paracetamol, ibuprofen, diclofenac, and naproxen (the former two do not form acyl glucuronides, while the latter three do) (Sánchez-Borges et al., 2010).

Moreover, like many other NSAIDs, it has been shown that zomepirac can also undergo metabolic activation by an oxidative process, independent of acyl glucuronide formation, further complicating the situation (Chen et al., 2006). Diclofenac has been closely investigated in terms of the potential association between drug-induced hypersensitivity and exposure to oxidative and conjugated metabolites of the drug (Harrer et al., 2010). The authors of this work concluded that there is no evidence for an IgE-mediated effector mechanism based on haptentation of protein carriers in diclofenac hypersensitive patients. Thus, the involvement of metabolites in diclofenac hypersensitivity reactions could be excluded. Furthermore, studies of protein modifications from patients who did not exhibit drug-related hypersensitivity to diclofenac showed either single adducts or combinations of adducts involving *N*-acylations or acyl glucuronide glycation (Hammond et al., 2014), indicating that adduct formation is not causative of hypersensitivity.

In the case of another NSAID, zomepirac, a similar analysis again failed to reveal a consistent increased risk for hypersensitivity relative to other members of this drug class. In a large patient analysis (Strom et al., 1987), NSAIDs were associated with an adjusted relative risk (95% confidence interval) of hypersensitivity reactions of 2.0. The increased risk was highest in those with acute pain (3.6) and absent in those without such a diagnosis [1.1 (0.6–1.9)]. For those exposed to zomepirac, the relative risk was 2.0. Stratification by the probable indication for NSAID usage suggested that the risk may be explained by the use of the NSAIDs for different indications. The authors concluded that the use of zomepirac appears to be associated with an increased risk compared with the use of other NSAIDs. However, the increased risk may be a function of the primary indication for the drug, or more likely the regimen associated with that indication rather than an intrinsic property of the drug.

Although withdrawn from the market due to its tendency to cause serious anaphylaxis in an unpredictable subset of the population, zomepirac is still being actively researched and recent publications have focused on kidney toxicity using mice pretreated with esterase and glutathione synthesis inhibitors (Iwamura et al., 2016). In these experiments, zomepirac acyl glucuronide concentrations in the kidney correlated with biomarkers of kidney injury. Extrapolation of the results to the clinical situation where zomepirac caused kidney injury should be judged against the pharmacological effects of cyclooxygenase inhibitors. Prostaglandins play a role in renal function, including vasodilatation, renin secretion, and sodium and water excretion. The result of cyclooxygenase inhibition may, therefore, be to disturb blood flow to the kidney, resulting in renal failure (Giovanni and Giovanni, 2002). Effects on the kidney, therefore, could be viewed as a class effect, the severity of which depends on individual patient status, potency of the agent, and clinical dose rather than on the formation of an acyl glucuronide.

Stratifying Acyl Glucuronide Stability and Reactivity. A number of approaches have been adopted to understand the relative risk of forming a chemically reactive acyl glucuronide. Iwamura et al. (2017) classified a set of acyl glucuronide-forming carboxylic acid drugs based on whether they had been withdrawn from the market ($n = 3$), carried black box warning labels ($n = 5$), or were considered safe ($n = 13$). A half-life of 3.6 hours or less in pH 7.4 aqueous buffer, pointing to increased reactivity, was suggested to be an indicator of heightened risk. Such stratification is useful, but should not be taken as evidence of safety or toxicity. The withdrawn toxic drugs included zomepirac, as previously discussed in detail. Also included was benoxaprofen, which was phototoxic to a high degree (as well as hepatotoxic). The phototoxicity was due to the parent compound and was a major factor in its withdrawal. Benoxaprofen is a good example of the need to understand clearance and metabolism pathways fully to design dosage regimens for safe use. This includes accounting for the major primary and secondary

metabolites. The initial drug half-life was around 24 hours in healthy volunteers (Nash et al., 1980), and a single dose of 600 mg was marketed. The skin toxicity and hepatotoxicity were observed almost exclusively in elderly patients (Halsey and Cardoe, 1982), subsequent studies in whom revealed a drug half-life extending out to 150 hours, which coincided with reduced creatinine clearance (Hamdy et al., 1982). Renal excretion of the major acyl glucuronide metabolite was believed to be the major clearance pathway. Enterohepatic recirculation of this metabolite and subsequent hydrolysis was also likely to be important in the persistence of the drug in the body. Gastrointestinal stasis and lowered kidney function, therefore, probably led to a marked accumulation of the drug in the elderly. Thus, the clearance of benoxaprofen by acyl glucuronidation was a more important factor in the toxicity of the drug rather than the reactivity of the acyl glucuronide.

Simplifying the Role of Species Differences. A Practical Approach to Qualifying Acyl Glucuronides of a Drug and Its Oxidative Metabolites. In this Commentary, we suggest that an analysis of the acyl glucuronidation of drugs and their oxidative metabolites should be conducted in the normal manner to establish relevant clearance pathways in the target patient population. However, for the purposes of qualifying metabolites in terms of metabolites in safety testing evaluation, we believe that the most practical and scientific qualification method is that acyl glucuronides of a drug and its oxidative metabolites should be considered collectively as a total system burden, rather than examined and qualified as individual metabolites.

In principle, the acylation and/or glycation of proteins by an acyl glucuronide conjugate can have two broad toxicological consequences, namely, modulation of protein function or production of an immunogen. Animal studies are only likely to detect the former (Cho and Utrecht, 2017). The rates of rearrangement/alkylation and acylation relate directly to the aqueous stability of the conjugates (Bailey and Dickinson, 2003; Sawamura et al., 2010; Iwamura et al., 2017). Importantly, the potential to acylate proteins is not dependent on fine structure remote from the site of conjugation, but relates to the overall size and shape of the total molecule and fine structure (steric hindrance, etc.) around the site of conjugation. The half-life of a number of acyl glucuronides in aqueous pH 7.4 potassium phosphate buffer, reported to be mainly a measure of susceptibility toward acyl migration, is shown in Table 1 (Sawamura et al., 2010; Zhang et al., 2011). The variation in structure remote from the carboxylic acid group is highly diverse but the conjugates show similar degrees of reactivity when classed according to the chemistry around the carboxylic acid moiety. Thus, the variation can be ascribed primarily to steric hindrance at the acyl moiety. This role of steric hindrance extends, as expected, to absolute stereochemistry, and the rates of acyl migration and hydrolysis are slightly different between structural isomers as exemplified by the *R* and *S* enantiomers of the 2-arylpropionic acid derivatives, where the α -methyl group is in very close proximity to the carboxylic acid. Although steric hindrance around the carboxyl group appears to be the major determinant of acyl glucuronide instability, the possibility that nearby oxidation may alter conjugate reactivity remains. For example, the acyl glucuronide of *S*-ibuprofen has a half-life of 3.68 hours in pH 7.4 buffer, whereas that for the corresponding conjugate of 2-hydroxyibuprofen is slightly longer at 5.03 hours (Johnson et al., 2007). At present, the only guideline to the risk posed by an acyl glucuronide is its stability/reactivity. This is a pragmatic concept, which could be termed a structural alert if the stability/reactivity is below a certain value. As outlined previously, a half-life of 3.6 hours or less in pH 7.4 aqueous buffer could be used as the basis for an alert (Iwamura et al., 2017).

There remains a need to establish some quantitative measure of metabolite safety to further define the risk-benefit relationship. Oxidative metabolites (and indeed stable *O*-ether- and *N*-glucuronide

TABLE 1

Half-lives of representative acyl glucuronide conjugates in aqueous buffer (pH 7.4) at 37°C

The data are adapted from Sawamura et al. (2010) and Zhang et al. (2011).

Drug	Chemical Description	Half-life in pH 7.4 Buffer hour
Diclofenac	Phenylacetic acid	0.7
Ibuprofen	Phenylacetic acid	0.9
Indomethacin	Indole acetic acid	1.6
Tolmetin	Pyrrrole acetic acid	0.4
Zomepirac	Pyrrrole acetic acid	0.5
Montelukast	Cyclopropane acetic acid	23.4
Benoxaprofen	Benzoxazole propionic acid	1.2
Ibuprofen	2-Phenylpropionic acid	3.0
Naproxen	2-Naphthylpropionic acid	2.3
Gemfibrozil	2,2-Dimethylvaleric acid	63
Flufenamic acid	Anthranilic acid	8.1
Furosemide	Anthranilic acid	4.1
Meclofenamic acid	Anthranilic acid	28.1
Mefenamic acid	Anthranilic acid	16.8
Probenicid	<i>Para</i> -substituted Benzoic acid	0.4
Repaglinide	2-Ethoxybenzoic acid	11.5
Telemisartan	Biphenyl-2-carboxylic acid	44.5
Muraglitazar	Phenylmethylaninoacetic acid	1.2
Peliglitazar	Phenylethylaminoacetic acid	10.1

conjugates) in the circulation or excreta should be considered by already well-established criteria. However, for acyl glucuronides, it seems prudent in qualification to ignore the various chemical forms of oxidative metabolites where the site of oxidation is distant from the carboxylic acid, and to sum the total acyl glucuronide burden derived from the drug. Thus, all acyl glucuronides of parent and metabolites in which the structure around the site of glucuronidation is essentially unaltered are considered collectively as a single amount or concentration. In the absence of reference standards of these conjugates (as is usually the case in early, or even late, development), semiquantitative or fit-for-purpose methods, as summarized in Luffer-Atlas and Atrakchi (2017), may be employed to estimate the total body burden of acyl glucuronides. This value then serves as the basis for qualifying acyl glucuronides of a drug of interest formed in humans relative to the corresponding exposure in animal species used in the preclinical safety assessment program.

As an example of this approach, early clinical development studies with the candidate antimalarial agent, P218 [3-(2-(3-((2,4-diamino-6-ethylpyrimidin-5-yl)oxyphenyl)propanoic acid)], demonstrated that the drug undergoes metabolism by oxidation of the phenyl ring to give a phenol (termed P218-OH) and by glucuronidation of the carboxylic acid moiety in both the parent and its phenolic metabolite (Fig. 1). The major drug-related compounds in human plasma following a single oral dose of P218 were the two acyl glucuronides, P218-acylgluc and P218-OH acylgluc, both of which were more abundant than the unchanged parent and exceeded 10% of total drug-related material, while levels of the free phenol were very low (Fig. 2). In contrast, preclinical safety studies demonstrated that the acyl glucuronide of P218 was the major circulating species in the rat, which formed only trace amounts of P218-OH and its acyl glucuronide, while in the dog, unchanged P218 was the most abundant metabolite in plasma, followed by P218-acylgluc; exposure to P218-OH and its acyl glucuronide were negligible in this species (Medicines for Malaria Venture, data on file). From the perspective of metabolites in safety testing, therefore, animal-to-human exposure margins could be demonstrated for both P218 and its acyl glucuronide conjugate based on the plasma area under the curve values in both rats and dogs. However, this was not true for P218-OH acylgluc, which thus was classified as a disproportionate major human metabolite.

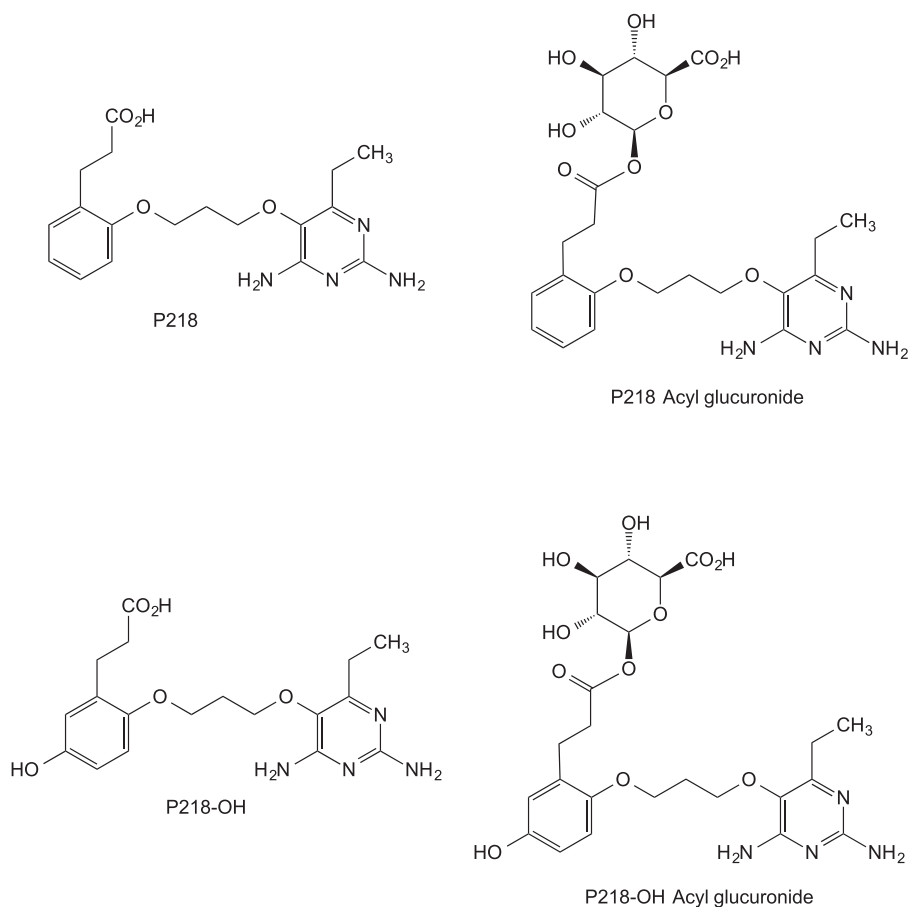


Fig. 1. Structures of P218 and P218 metabolites.

The problem, then, was how best to qualify P218-OH acylgluc in terms of its safety, given the considerable difficulties associated with the chemical synthesis and biologic evaluation of an inherently unstable metabolite? Application of the total body burden reasoning outlined previously, in which systemic exposure to all acyl glucuronides in each species is summed, provided a potential solution to this problem. As shown in Fig. 2, the sum of acyl glucuronide (total acylgluc) exposures in the dog was almost double that seen in human, while the corresponding plasma area under the curve sum in rats approximated the total acylgluc exposure in humans. Since the animal data for this analysis derived from doses corresponding to the respective no adverse effect level values in the preclinical safety studies, it could be concluded, therefore, that the major circulating metabolites of P218 in humans had been adequately qualified from a safety perspective.

As illustrated by this example, the total body burden approach allows for expected species differences in oxidation, glucuronidation, and glucuronide hydrolysis, and while not perfect the summation provides a good estimate of potential safety risk as reflected by animal-to-human exposure margins. One caveat here would be if the parent drug were to inhibit Mrp transporters, thereby impairing biliary elimination of acyl glucuronide conjugates, potentially in a species-dependent manner, then this approach may need to be modified. An additional factor that merits consideration is the dose of the parent carboxylic acid, since it is known that high-dose drugs of this class traditionally have a poorer safety record than their low-dose counterparts (Smith and Schmid, 2006).

The Unsatisfactory or Impractical Alternatives. One alternative to addressing an unqualified acyl glucuronide in humans is to abandon a potentially valuable medicine—clearly, an unattractive proposition. The alternatives, however, in fully qualifying such a metabolite by traditional

approaches, could be technically and scientifically very challenging to the extent that the risks involved and the uncertainty could lead to the same unattractive conclusion.

The instability of acyl glucuronides in aqueous solution poses formidable challenges in establishing appropriate tests for an unqualified acyl glucuronide, whether it is a conjugate of the parent drug itself or an oxidative metabolite thereof. Isolation or chemical synthesis of acyl glucuronides is possible, but extremely difficult in any quantity

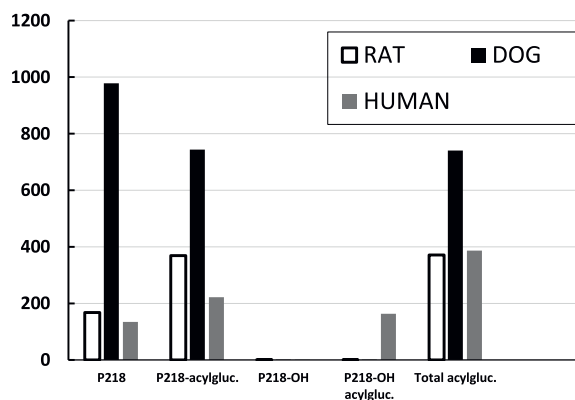


Fig. 2. Area under the curve (AUC; $\mu\text{g}\cdot\text{hr}/\text{ml}$) values at the no adverse effect level doses in animals (100 mg/kg rat; 20 mg/kg dog) compared with the clinical dose (10 mg/kg) for P218, an antimalarial agent designed for single-dose administration and cure. The AUC values are the cumulative AUCs over 14-day toxicology studies compared with the $\text{AUC}_{0-\text{inf}}$ after a single dose in human. The values shown are for the individual metabolites and the total for acyl glucuronides.

necessary for testing. Both in vitro and in vivo studies would be at risk of being severely compromised by the formation of parent acid (through hydrolysis) and ring migrated isomers of the conjugate. The route of administration of a preformed acyl glucuronide would have to be systemic, which runs the risk of producing misleading results compared with the real life situation where conjugation of the parent and any phase I metabolites takes place in the gut wall and/or liver (Pruksaritanont et al., 2006). For these reasons, the simplified paradigm outlined in this Commentary is advanced as a practical approach to assessing the safety of acidic drugs that undergo metabolism via acyl glucuronidation and other routes, and exhibit species differences in the various forms circulating and present in excreta.

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Authorship Contributions

Participated in research design: Smith, Hammond, Baillie.

Wrote or contributed to the writing of the manuscript: Smith, Hammond, Baillie.

References

- Bailey MJ and Dickinson RG (2003) Acyl glucuronide reactivity in perspective: biological consequences. *Chem Biol Interact* **145**:117–137.
- Chen Q, Doss GA, Tung EC, Liu W, Tang YS, Braun MP, Didolkar V, Strauss JR, Wang RW, Stearns RA, et al. (2006) Evidence for the bioactivation of zomepirac and tolmetin by an oxidative pathway: identification of glutathione adducts in vitro in human liver microsomes and in vivo in rats. *Drug Metab Dispos* **34**:145–151.
- Cho T and Utrecht J (2017) How reactive metabolites induce an immune response that sometimes leads to an idiosyncratic drug reaction. *Chem Res Toxicol* **30**:295–314.
- Faed EM (1984) Properties of acyl glucuronides: implications for studies of the pharmacokinetics and metabolism of acidic drugs. *Drug Metab Rev* **15**:1213–1249.
- Giovanni G and Giovanni P (2002) Do non-steroidal anti-inflammatory drugs and COX-2 selective inhibitors have different renal effects? *J Nephrol* **15**:480–488.
- Halsey JP and Cardoe N (1982) Benoxaprofen: side-effect profile in 300 patients. *Br Med J (Clin Res Ed)* **284**:1365–1368.
- Hamdy RC, Murnane B, Perera N, Woodcock K, and Koch IM (1982) The pharmacokinetics of benoxaprofen in elderly subjects. *Eur J Rheumatol Inflamm* **5**:69–75.
- Hammond TG, Meng X, Jenkins RE, Maggs JL, Castelazo AS, Regan SL, Bennett SN, Earnshaw CJ, Aithal GP, Pande I, et al. (2014) Mass spectrometric characterization of circulating covalent protein adducts derived from a drug acyl glucuronide metabolite: multiple albumin adductions in diclofenac patients. *J Pharmacol Exp Ther* **350**:387–402.
- Harrer A, Lang R, Grims R, Braitsch M, Hawranek T, Aberer W, Vogel L, Schmid W, Ferreira F, and Himly M (2010) Diclofenac hypersensitivity: antibody responses to the parent drug and relevant metabolites. *PLoS One* **5**:e13707.
- Iwamura A, Nakajima M, Oda S, and Yokoi T (2017) Toxicological potential of acyl glucuronides and its assessment. *Drug Metab Pharmacokin* **32**:2–11.
- Iwamura A, Watanabe K, Akai S, Nishinosono T, Tsuneyama K, Oda S, Kume T, and Yokoi T (2016) Zomepirac acyl glucuronide is responsible for zomepirac-induced acute kidney injury in mice. *Drug Metab Dispos* **44**:888–896.

- Johnson CH, Wilson ID, Harding JR, Stachulski AV, Iddon L, Nicholson JK, and Lindon JC (2007) NMR spectroscopic studies on the in vitro acyl glucuronide migration kinetics of Ibuprofen ((±)-(R,S)-2-(4-isobutylphenyl) propanoic acid), its metabolites, and analogues. *Anal Chem* **79**:8720–8727.
- Liu JH and Smith PC (2006) Predicting the pharmacokinetics of acyl glucuronides and their parent compounds in disease states. *Curr Drug Metab* **7**:147–163.
- Luffer-Atlas D and Atrakchi A (2017) A decade of drug metabolite safety testing: industry and regulatory shared learning. *Expert Opin Drug Metab Toxicol* **13**:897–900.
- Monrad RN, Errey JC, Barry CS, Iqbal M, Meng X, Iddon L, Perrie JA, Harding JR, Wilson ID, Stachulski AV, et al. (2014) Dissecting the reaction of phase II metabolites of ibuprofen and other NSAIDs with human plasma protein. *Chem Sci* **5**:3789–3794.
- Nanau RM and Neuman MG (2010) Ibuprofen-induced hypersensitivity syndrome. *Transl Res* **155**:275–293.
- Nash JF, Carmichael RH, Ridolfo AS, and Spradlin CT (1980) Pharmacokinetic studies of benoxaprofen after therapeutic doses with a review of related pharmacokinetic and metabolic studies. *J Rheumatol Suppl* **6** (Suppl):12–19.
- Ogilvie BW, Zhang D, Li W, Rodrigues AD, Gipson AE, Holsapple J, Toren P, and Parkinson A (2006) Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos* **34**:191–197.
- Pruksaritanont T, Lin JH, and Baillie TA (2006) Complicating factors in safety testing of drug metabolites: kinetic differences between generated and preformed metabolites. *Toxicol Appl Pharmacol* **217**:143–152.
- Regan SL, Maggs JL, Hammond TG, Lambert C, Williams DP, and Park BK (2010) Acyl glucuronides: the good, the bad and the ugly. *Biopharm Drug Dispos* **31**:367–395.
- Sánchez-Borges M, Caballero-Fonseca F, Capriles-Hulett A, and González-Aveledo L (2010) Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs: an update. *Pharmaceuticals (Basel)* **3**:10–18.
- Sawamura R, Okudaira N, Watanabe K, Murai T, Kobayashi Y, Tachibana M, Ohnuki T, Masuda K, Honma H, Kurihara A, et al. (2010) Predictability of idiosyncratic drug toxicity risk for carboxylic acid-containing drugs based on the chemical stability of acyl glucuronide. *Drug Metab Dispos* **38**:1857–1864.
- Shipkova M, Armstrong VW, Oellerich M, and Wieland E (2003) Acyl glucuronide drug metabolites: toxicological and analytical implications. *Ther Drug Monit* **25**:1–16.
- Shitara Y, Hirano M, Sato H, and Sugiyama Y (2004) Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* **311**:228–236.
- Smith DA and Schmid EF (2006) Drug withdrawals and the lessons within. *Curr Opin Drug Discov Devel* **9**:38–46.
- Smith PC, Benet LZ, and McDonagh AF (1990) Covalent binding of zomepirac glucuronide to proteins: evidence for a Schiff base mechanism. *Drug Metab Dispos* **18**:639–644.
- Spahn-Langguth H and Benet LZ (1992) Acyl glucuronides revisited: is the glucuronidation process a detoxification as well as a detoxification mechanism? *Drug Metab Rev* **24**:5–47.
- Stachulski AV (2011) Chemistry and reactivity of acyl glucuronides. *Curr Drug Metab* **12**:215–221.
- Strom BL, Carson JL, Morse ML, West SL, and Soper KA (1987) The effect of indication on hypersensitivity reactions associated with zomepirac sodium and other nonsteroidal anti-inflammatory drugs. *Arthritis Rheum* **30**:1142–1148.
- Tornio A, Filppula AM, Kailari O, Neuvonen M, Nyrönen TH, Tapaninen T, Neuvonen PJ, Niemi M, and Backman JT (2014) Glucuronidation converts clopidogrel to a strong time-dependent inhibitor of CYP2C8: a phase II metabolite as a perpetrator of drug-drug interactions. *Clin Pharmacol Ther* **96**:498–507.
- Zhang D, Raghavan N, Wang L, Xue Y, Obermeier M, Chen S, Tao S, Zhang H, Cheng PT, Li W, et al. (2011) Plasma stability-dependent circulation of acyl glucuronide metabolites in humans: how circulating metabolite profiles of muraglitazar and pelgilitazar can lead to misleading risk assessment. *Drug Metab Dispos* **39**:123–131.

Address correspondence to: Thomas A. Baillie, Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Box 357610, Seattle, WA 98195-7610. E-mail: tbaille@uw.edu