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Mini-Review

Acyl glucuronide metabolites: Implications for drug safety assessment

Terry R. Van Vleet[a,*], Hong Liu[b], Anthony Lee[b], Eric A.G. Blomme[a]

[a] Abbvie, Development Sciences, Department of Preclinical Safety, United States
[b] Abbvie, Development Sciences, Biomeasure and Metabolism, United States

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ABSTRACT

Acyl glucuronides are important metabolites of compounds with carboxylic acid moieties and have unique properties that distinguish them from other phase 2 metabolites. In particular, in addition to being often unstable, acyl glucuronide metabolites can be chemically reactive leading to covalent binding with macromolecules and toxicity. While there is circumstantial evidence that drugs forming acyl glucuronide metabolites can be associated with rare, but severe idiosyncratic toxic reactions, many widely prescribed drugs with good safety records are also metabolized through acyl glucuronidation. Therefore, there is a need to understand the various factors that can affect the safety of acyl glucuronide-producing drugs including the rate of acyl glucuronide formation, the relative reactivity of the acyl glucuronide metabolite formed, the rate of elimination, potential proteins being targeted, and the rate of aglucuronidation. In this review, these factors are discussed and various approaches to de-risk the safety liabilities of acyl glucuronide metabolites are evaluated.

1. Introduction

Many compounds containing carboxylic acid functional groups are metabolized mostly in the liver through the uridine diphosphate (UDP) glucuronosyl transferase (UGT) enzyme family in addition to other metabolic pathways leading to the generation of metabolites such as acyl-coenzyme A thioester (Skonberg et al., 2008). The UGT-mediated metabolism pathway is considered quantitatively the most important for carboxylic acids and leads to the formation of acyl glucuronide metabolites, which are more polar than the parent compound with the hydrophilic glucuronic acid moiety. Multiple marketed and commonly prescribed drugs are known to produce acyl glucuronide metabolites, including a variety of nonsteroidal anti-inflammatory drugs (NSAIDs), diuretics, anticonvulsants, antibiotics, and hypolipidemic agents (e.g., fibrates) (Wen et al., 2007).

Acyl glucuronide metabolites have unique intrinsic properties that can make them of significant concern from a toxicological perspective. In particular, and in contrast to most phase 2 metabolites, which are typically inactive and rapidly excreted from the body, some acyl glucuronides can be unstable *in vitro* and *in vivo* under physiological conditions. More importantly, acyl glucuronides are intrinsically reactive; they can undergo hydrolysis, rapid intramolecular rearrangement and interact with normal cellular macromolecules (e.g., proteins, DNA, lipids) through covalent binding by transacylation or glycation (Skonberg et al., 2008; Miyashita et al., 2014; Thompson et al., 2016). Intrinsic reactivity is particularly important, since reactive metabolites and covalent binding have been associated with idiosyncratic toxic reactions, especially idiosyncratic hepatotoxicity (Park et al., 1992; Skonberg et al., 2008; Sawamura et al., 2010; Scialis and Manautou, 2016; Thompson et al., 2016). This has led some scientists in the pharmaceutical industry to argue that compounds with a

Abbreviations: UGT, UDP glucuronyl transferase; UDP, uridine diphosphate; KPB, potassium phosphate buffer; HLM, human liver microsomes; IDT, idiosyncratic drug toxicity; HSA, human serum albumin; GSH, glutathione; NSAID, nonsteroidal anti-inflammatory drug; GSH, glutathione; hERG, human Ether-à-go-go-Related gene; CNS, central nervous system; MRP, multidrug resistance associated protein; CYP, cytochrome P450; USFDA, United States Food and Drug Administration; MIST, metabolites in safety testing; DILI, drug induced liver injury.

* Corresponding author at: Abbvie Bioresearch Center, 1 Waukegan Road, North Chicago, IL, United States.
E-mail address: terry.vanvleet@abbvie.com (T.R. Van Vleet).

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carboxylic acid moiety should be avoided when developing new small molecule-based medicines (Stepan et al., 2011; Stachulski and Meng, 2013; Charifson and Walters, 2014).

Carboxylate moieties, however, can be an important pharmacophore for the medicinal chemist. Acids are generally dissolved to a greater extent in the small intestine where pH is favorable to ionized species (Bocker et al., 2010). Other attractive properties of carboxylic acids as drug candidates include increased diversity of clearance mechanisms (introducing non-CYP mediated metabolism and biliary clearance, therefore, reducing CYP-mediated drug-to-drug interaction risk) and negatively charged moieties that can participate in salt bridges (Bocker et al., 2010). The ability to form strong electrostatic interactions and hydrogen bonds is generally the major reason that carboxylic acids are selected as a functional group for drug-target interactions (Ballatore et al., 2013). The presence of negatively charged moieties, such as carboxylic acids, can also be used to prevent interaction with the hERG (human Ether-à-go-go-Related; alpha subunit of a potassium channel) channel. hERG inhibition can result clinically in delayed cardiac repolarization and prolongation of the QT interval (interval between the Q and T waves of an ECG trace), as well as potential lethal cardiac arrhythmia know as Torsades de Pointes (Gleeson, 2008; Rampe and Brown, 2013). However, charged compounds generally tend to have poor permeability and higher albumin binding, which can limit the volume of distribution and lower exposure to free drug (Zsila et al., 2011).

The objective of this short review is to evaluate the available evidence associating acyl gluycuronide metabolites to toxicity issues and to evaluate approaches to support the development of safe carboxylic acid-based compounds as pharmaceutical agents.

2. Acyl glucuronidation as an excretion pathway

Acyl glucuronide metabolites are formed by the glucuronidation of carboxylate moieties of drugs, xenobiotics, or endogenous substances, such as bilirubin, bile and fatty acids (Sawamura et al., 2010). Fig. 1 summarizes the formation and disposition of acyl glucuronide metabolites *in vivo*. Glucuronidation results in the attachment of glucuronic acid to a nucleophilic center of the substrate molecule catalyzed by the UGTs, a family of enzymes that are membrane-bound and found in the endoplasmic reticulum of multiple tissues, especially the liver (Sakaguchi et al., 2004; Skonberg et al., 2008). Glucuronidation is a common phase 2 metabolic reaction and UGTs are among the most important

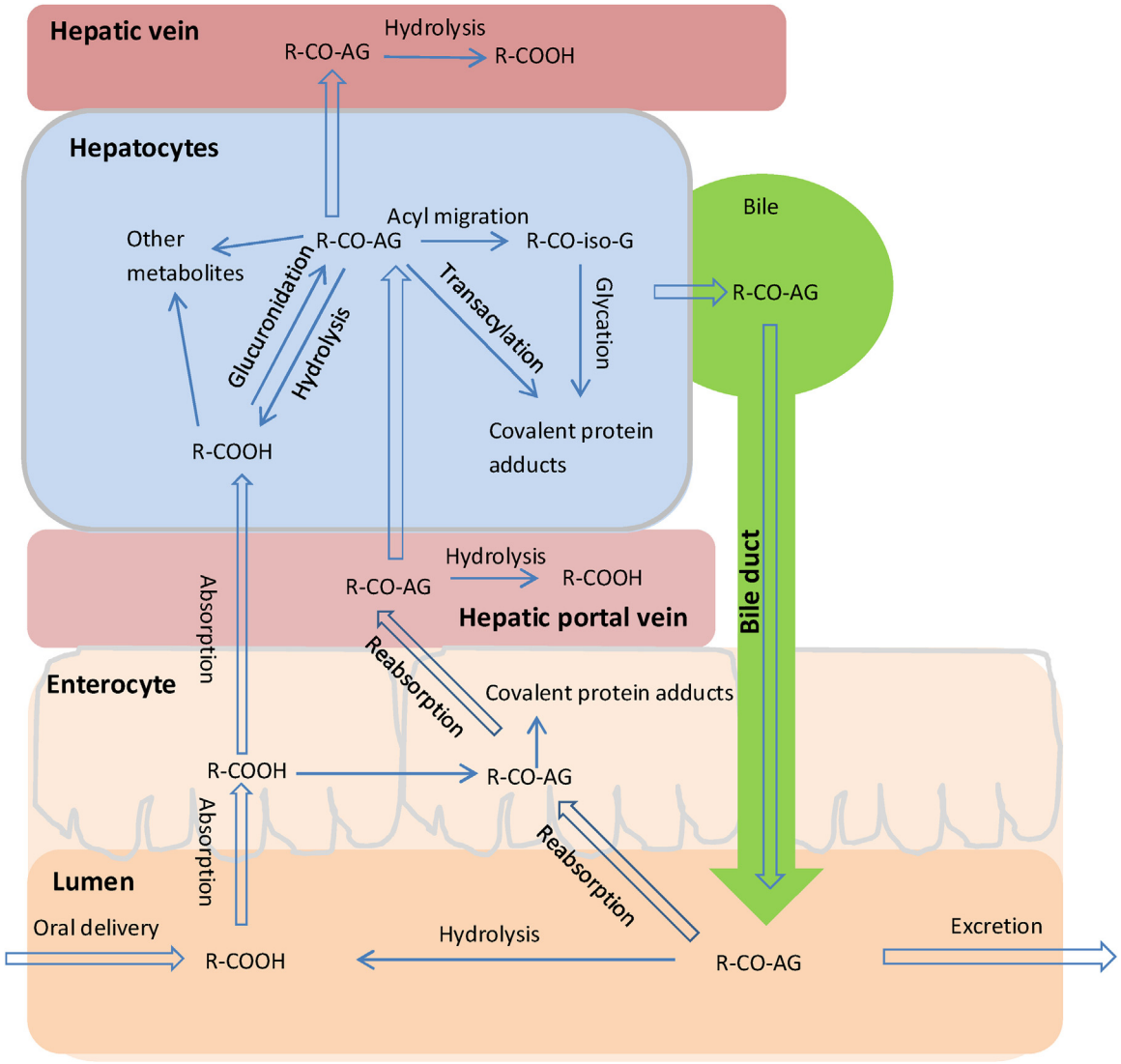

Fig. 1. Metabolism and disposition of carboxylic acids containing drugs and their acyl glucuronide metabolites following oral exposure. R-COOH represents parent drug and R-CO-AG represents acyl glucuronide drug metabolite.