Are drugs containing a carboxylic acid functional group associated with a significant risk of idiosyncratic drug reactions?

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Abstract: Most drugs that contain a carboxylic acid are metabolized to acyl glucuronides. Although the reactivity of these metabolites varies, acyl glucuronides can clearly react with proteins. There is circumstantial evidence that many idiosyncratic drug reactions are caused by reactive metabolites; therefore, it is quite plausible that the reactive metabolites of carboxylic acids would cause such reactions. However, not all drugs that form reactive metabolites are associated with a significant risk of idiosyncratic reactions. In fact, the carboxylic acid functional group is common, and in most cases the drugs are quite safe. The largest problem is with nonsteroidal antiinflammatory drugs, and much of the associated adverse reactions are related to their pharmacological action. In most other cases there is an alternative reactive metabolite. This raises the question as to why the reactive acyl glucuronides are not associated with a higher risk of idiosyncratic reactions. It appears that for a drug to provoke an immune response it must do two things: form a neoantigen and cause cell damage. It is possible that the amide bond formed when acyl glucuronides react with proteins is cleaved when antigens are processed, which also involves cleavage of amide bonds. Given that acyl glucuronides are less reactive than most reactive metabolites formed by oxidation, they may also not cause significant cell damage. It is possible that some idiosyncratic reactions are caused by reactive metabolites of carboxylic acids; however, overall the carboxylic acid functional group is very important and is generally not associated with a significant risk.

Keywords: Acyl glucuronides, Co-A esters, Idiosyncratic drug reactions, Immune mediated, Reactive metabolites, Adverse drug reactions.

1. INTRODUCTION

The two major issues that lead to failure of a drug candidate are lack of efficacy and adverse reactions. Some types of adverse reactions are related to the therapeutic effect of the drug and impossible to avoid. Other effects can be toxicities that are detected in routine animal testing or even *in vitro* assays. The most difficult type of adverse reaction to deal with is an idiosyncratic drug reaction (IDR). Such reactions are usually only detected late in phase III clinical trials or even after the drug has been released onto the market. This markedly increases the risk of drug development. If there were accurate methods to predict the risk that a drug candidate is likely to cause an unacceptable risk of IDRs it would have a profound effect on the process of drug development. Many attempts have been made to devise methods to predict the risk of IDRs; however, there is no good evidence that any method is accurate. One simple method is to avoid "structural alerts". Structural alerts are chemical structures that are associated with a high risk of IDRs. In most cases this is because these structures are chemically reactive or can readily be converted to a chemically reactive metabolite. The objective of this paper is to examine the evidence that carboxylic acids should be considered a structural alert.

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2. IDR MECHANISMS

IDRs are very difficult to study. In theory, the best way to study the mechanism of an IDR would be to obtain samples from a patient before the onset of the IDR to determine what biological events led to the adverse effect. However, given the unpredictable nature of IDRs, that is virtually impossible. To study patients after the onset of an IDR is like the study of a plane crash without the aid of the "black box"; it is difficult to know what is cause and what is effect.

Animal models are important for the study of biological processes, but IDRs are also idiosyncratic in animals. Although *in vitro* studies can examine specific biological effects, they cannot be expected to represent the complexity of an IDR. Therefore, it is very difficult to be confident that a specific effect found in such assays is actually part of the mechanism of an IDR unless it can be shown that the effect occurs in patients who experience an IDR, and it predicts who will experience an IDR. The early events in an IDR appear to be mediated by the innate immune system, which are clinically silent but not idiosyncratic, and therefore amenable to study in both animals and humans [1]. But at the present time, we are left with inferring mechanism from the clinical characteristics of IDRs and factors that affect the risk of an IDR.

The risk factors can relate to characteristics of patients who actually develop IDRs and also to the features of drugs that cause IDRs. The most

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compelling patient characteristic that is linked to the risk that a patient will have an IDR when treated with a specific drug is human leukocyte antigen (HLA) haplotype. For example, an early finding was a strong association between HLA-B*1502 and the risk of carbamazepine-induced toxic epidermal necrolysis [2]. Since then several other associations between specific HLA haplotypes and the risk of an IDR have been found [3], and the list continues to grow. The association of IDRs with specific HLA haplotypes strongly suggests these IDRs are immune mediated. HLA stands for human lymphocyte antigen. This is the human version of the major histocompatibility antigen (MHC). This comes in two versions; MHC-I is involved in presenting antigen to T cell receptors on CD8 T cells, and MHC-II is involved in presenting antigen to T cell receptors on CD4 T cells. However, most IDRs have not been linked to a specific HLA haplotype. In most cases this is likely because there were not sufficient patients available with a specific IDR to study. It is also possible that some reactive metabolites modify so many proteins that most patients will have one HLA that can "recognize" one of the very large number of drug-modified peptides. Many other genetic associations with the risk of IDRs also involve the immune system. These include protein tyrosine phosphatase non-receptor 22 (PTPN22) [4] and interleukin 10 (IL-10) [5].

The clinical characteristics of IDRs, including their idiosyncratic nature and histology, are also strong evidence for an immune mechanism [6]. In particular, the histology of some of the most serious IDRs such as hepatocellular drug-induced liver injury [7] and toxic epidermal necrolysis [8] is dominated by CD8 T cells, which provides strong evidence that these IDRs are immune mediated. Although tissue injury can provoke an immune response to repair the injury, the major function of CD8 T cells is to kill virus-infected cells or cancer cells, and their presence is very unlikely to represent a repair process. Obviously, autoimmune IDRs are immune mediated. These IDRs can either involve one organ such as drug-induced autoimmune hepatitis, or they can be a more generalized autoimmune reaction such as a lupus-like IDR [9]. Although the evidence for an immune mechanism for many IDRs is very strong, given the complexity of biological systems, it is quite possible that some IDRs are not immune mediated. However, at present, there is little clinical evidence for alternative mechanisms, and *in vitro* studies, on their own, cannot be relied on to provide conclusive evidence for the mechanism of a clinical adverse reaction.

3. ROLE OF REACTIVE METABOLITES IN THE MECHANISM OF IDRS

The feature of drugs that is most closely associated with an increased risk of IDRs is the formation of a reactive metabolite, or in a few cases such as penicillin, the drug itself is chemically reactive. However, it is very difficult to prove that a specific reactive metabolite of a drug is responsible for an IDR. There are only a few examples where we can be certain that a reactive species is responsible for an IDR. One example is penicillin-induced anaphylaxis. Penicillin is chemically reactive because of its ß-lactam ring, which is responsible for covalent binding to proteins. Penicillin-induced anaphylaxis is mediated by IgE antibodies against penicillin-modified proteins. Therefore, we can be confident that the mechanism of penicillin-induced anaphylaxis involves covalent binding of the drug to proteins, which in a few patients leads to the formation of IgE antibodies against the drug-modified proteins. Another example is nevirapine-induced skin rash. Nevirapine causes an immune mediated skin rash in female Brown Norway rats that is very similar to the rash it causes in humans. Nevirapine forms a reactive benzylic sulfate in the skin, and a topical sulfotransferase inhibitor prevents covalent binding and rash where it is applied [10]. This provides very compelling evidence that the rash is due to this chemically reactive sulfate metabolite. It was only possible to perform such controlled experiments because we had an animal model very similar to the IDR that occurs in humans, but this is rare. There are other examples such as halothane-induced liver injury in which the evidence is strong, but it falls short of being conclusive. Specifically, halothane is a relatively inert molecule, but it is oxidized to a very reactive acid chloride, which covalently binds to proteins. Isoflurane, which is oxidized to the same reactive metabolite but to a much lesser degree, is associated with a decreased risk of liver injury [11]. Patients with halothane-induced hepatitis have antibodies against trifluoroacetylated proteins [12]; however, unlike the case of penicillin-induced anaphylaxis, it is not clear what role these antibodies play in the injury. The antibodies simply indicate that binding of the reactive metabolite has provoked an immune response, but it is more likely that most of the liver damage is mediated by CD8 T cells.

On the other hand, there are some drugs that form a significant amount of reactive metabolite but are not associated with a significant risk of IDRs. For example, ethacrynic acid is a chemically reactive Michael acceptor, which covalently binds to proteins and forms

a glutathione conjugate [13]. However, it is not associated with a significant risk of IDRs. Olanzapine forms a reactive metabolite very similar to that of clozapine [14], and yet it is not associated with the same risk of agranulocytosis. One difference between clozapine and olanzapine is dose; however, even at the same dose, the two drugs have different effects on neutrophil kinetics in rats [15]. And there are other drugs that are associated with a relatively high risk of IDRs that do not appear to form a reactive metabolite. Examples include, ximelagatran, pyrazinamide, and allopurinol. Although it has been reported that there is a correlation between the amount of reactive metabolite formation (corrected for dose) and the risk of idiosyncratic drug-induced liver injury [16], others have not found such a simple relationship [17, 18]. In summary, it appears likely that reactive metabolites play a role in the mechanism of many, if not most IDRs, but the evidence is not conclusive, and there are likely exceptions.

If reactive metabolites are not always associated with a significant risk of IDRs, there must be characteristics of the reactive metabolite other than covalent binding that are relevant to IDR risk. An obvious characteristic is reactivity. Most reactive metabolites are sufficiently reactive that they do not reach sites distant from where they are formed. That is presumably one reason the liver is a common target of IDRs; it is where most drug metabolism occurs and reactive metabolites are formed. A major class of enzymes responsible for reactive metabolite formation is the cytochromes P450. Some reactive metabolites are so reactive that most of the binding is to the enzyme that formed them. This can lead to so-called mechanism-based inhibition of the enzyme [19]. In contrast, a few reactive metabolites or drugs such as penicillin have relatively low reactivity and freely circulate. Another characteristic is whether the reactive metabolite can redox cycle, i.e. after being oxidized to a chemically reactive species, it can be reduced back to the parent drug, often along with the production of reactive oxygen species. Another consideration is whether the reactive metabolite is a “hard” or “soft” electrophile [20]. Hard reactive metabolites bind to hard nucleophiles such as amino groups, while soft reactive metabolites bind mostly to soft nucleophiles such as a cysteine thiol. However, even metabolites with similar half-lives and “hardness” bind to different proteins. For example, the reactive metabolites of clozapine and vesnarinone are both “soft” so they react with cysteines, and they also have similar half-lives, but they bind to a very different spectrum of proteins [21]. This is presumably because of noncovalent interactions between the metabolite and protein that precede the covalent binding. Where the reactive metabolite is formed is also presumably important. As mentioned, most drug metabolism occurs in the liver, and the liver is a major target of IDRs. However, as also mentioned, there is sulfotransferase in the skin that can lead to the formation of reactive metabolites. Leukocytes, such as neutrophils, monocytes, and their precursors, have myeloperoxidase that can oxidize some drugs such as clozapine to a reactive metabolite, and clozapine can cause agranulocytosis, i.e. a lack of neutrophils [22].

A fundamental question is: how do reactive metabolites cause IDRs? Two complementary hypotheses are the hapten and danger hypotheses [23]. The covalent binding of a reactive metabolite to proteins produces neoantigens that can be viewed as “foreign” by the immune system. This represents the classic hapten hypothesis. However, it appears that simply being foreign is not sufficient to induce an immune response; the foreign protein must also be associated with causing some type of cell damage; this is the danger hypothesis [24]. Cell damage leads to the release of danger-associated molecular pattern molecules (DAMPs), which activate antigen presenting cells [23]. All reactive metabolites can act as haptens, but not all reactive metabolites lead to significant cell damage, and this may differentiate reactive metabolites that cause IDRs from those that do not. It is likely that it is more complex than this. For example, some drugs also inhibit other important pathways such as the bile salt export protein (BSEP). Such inhibition may cause cell stress that is independent of reactive metabolite formation, and this may contribute to the ability of a drug to cause an IDR [25].

4. STRUCTURAL ALERTS

Some functional groups are readily metabolized to reactive metabolites and are referred to as structural alerts. A classic example is a primary aromatic amine. This functional group is readily metabolized to several reactive metabolites. These include a hydroxylamine, which can be further metabolized by *O*-acetylation or sulfation, a nitroso metabolite, and several free radial intermediates. Early studies into the mechanism of chemical carcinogenesis focused on the bioactivation of aromatic amine carcinogens that react with DNA [26]. Aromatic nitro groups are reduced to the same reactive intermediates as are formed by oxidation of aromatic amines. Other functional groups that often

form reactive metabolites are hydrazines, thiono sulfur compounds, thiophenes, and furans [27]. If IDRs could be avoided by simply avoiding structural alerts, it would certainly simplify drug development. However, there are many pathways leading to reactive metabolites that are more complex and are not captured by focusing on structural alerts. In addition, not all drugs that possess these functional groups are associated with a relatively high risk of IDRs. Furthermore, as mentioned earlier, it is almost impossible to be certain that a specific functional group or reactive metabolite is responsible for a specific IDR. One example is carboxylic acids.

5. THE CASE OF CARBOXYLIC ACIDS

Carboxylic acids are often considered to be structural alert, but what is the evidence? They can form two types of reactive metabolite, acyl glucuronides and acyl-Co-A thioesters. These metabolites can clearly covalently bind to proteins; therefore, it is quite plausible that such metabolites could be responsible for IDRs.

5.1. Acyl Glucuronides

The major metabolic pathway for most carboxylic acids is the formation of an acyl glucuronide. Although glucuronidation is an important route of clearance for drugs and other chemicals, acyl glucuronides are chemically reactive [28]. These metabolites can react with proteins by two different mechanisms [29]. The most straightforward mechanism is nucleophilic attack on the carbonyl carbon with displacement of the glucuronic acid. The nucleophile is usually an amino group, but acyl glucuronides can also react with alcohols such as serine and thiols such as glutathione [30]. The product of the reaction with glutathione is also reactive as described below, but it is readily hydrolyzed and has a short half-life. The other mechanism by which an acyl glucuronide can lead to covalent binding involves migration of the acyl group around the glucuronic acid molecule, followed by ring opening to form a reactive aldehyde group, reaction of the aldehyde with a protein amino group to form a Schiff base, and finally an Amadori rearrangement to form a very stable covalent bond [29]. In this case the glucuronic acid moiety remains part of the product. However, a study in which they were unable to trap the putative aldehyde intermediate with several drugs that form acyl glucuronides and are associated with IDRs suggests that this is not a major pathway leading to covalent binding and IDRs [31]. Given the association between the formation of reactive metabolites and the risk that a drug will cause an unacceptable risk of IDRs, it is quite plausible that acyl glucuronides can be responsible for IDRs. Guidance documents from the US Food and Drug Administration as recently as 2020 suggest that acyl glucuronides are potentially toxic; therefore, carboxylic acids require special consideration. The reactivity of acyl glucuronides varies considerably [32]. Several attempts have been made to relate the reactivity of the acyl glucuronide with the risk of IDRs as discussed below [33, 34].

5.2. Acyl-Co-A Thioesters

Carboxylic acids can form acyl coenzyme-A (Co-A) thioesters, which are also chemically reactive. This pathway is the first step in the metabolism of fatty acids, and it can also lead to amino acid conjugation of benzoic acids and their analogs and chiral inversion of 2-arylpropionic acids [35]. Acyl Co-A thioesters do not reach significant concentrations outside of the liver; therefore, it is unlikely that they would cause IDRs outside of the liver. However, as mentioned, acyl glucuronides can also react with thiol-containing molecules such as glutathione to form reactive species similar to the Co-A thioesters, and this could occur outside of the liver.

6. WHAT IS THE CLINICAL EVIDENCE THAT CARBOXYLIC ACIDS ARE A STRUCTURAL ALERT?

A significant problem with studies that try to infer mechanism from correlation with risk is that it is very difficult to accurately determine IDR risk. In the calculation of risk, one needs to know both the number of cases of adverse reactions and the number of individuals exposed for a sufficient period of time to be at risk. Specifically, depending on the type of IDR, most have a delay in onset of weeks for rashes, months for liver injury, and often more than a year for autoimmune type IDRs; therefore, patients exposed to a drug for a short period of time would not be at risk. Only about 10% of serious IDRs are reported [36], but it varies significantly between different drugs, and causality is often uncertain when a case is reported. Databases such as the FAERS database are especially problematic. Having reviewed many FAERS adverse event reports, there was so much data missing that I found it difficult to determine what the adverse event was, let alone what caused it. Drugs that have been withdrawn from the market are also problematic because there is hardly any clinical data available to evaluate the adverse reactions associated with drugs

such as ibufenac, fenclofenac, and isoxepac, and there is also little known about their metabolites. The best source of information about the risk of idiosyncratic drug-induced liver injury (IDILI) is probably LiverTox (https://www.ncbi.nlm.nih.gov/books/NBK547852/), which is produced by the National Institute of Diabetes and Digestive Diseases. There are also good reviews on drugs associated with a significant incidence of serious skin rashes [37-39].

In the paper by Sawamura *et al*. drugs containing a carboxylic acid functional group are categorized as safe, warning, or withdrawn, and the claim is that this is clearly associated with the reactivity of the acyl glucuronide [34]. Included in the safe category are levofloxacin and meclofenamate. But the LiverTox website lists levofloxacin as a clear cause of IDILI, and fluoroquinolones are a probable cause of toxic epidermal necrolysis [39]. Meclofenamate is more commonly used in horses; it is seldom used in humans because of gastrointestinal side-effects, but there are convincing cases of thrombocytopenia with a positive rechallenge [40] and agranulocytosis [41].

Drugs included in the warning category are diclofenac, ibuprofen, indomethacin, mefenamic acid, naproxen, and tolmetin. This implies that they all associated with a similar level of IDR risk, and all are listed as causing anaphylaxis. All of these drugs are nonsteroidal antiinflammatory drugs (NSAIDs), which inhibit the synthesis of arachidonic acid and increase the production of leukotrienes. This can lead to a pseudoallergic reaction that is related to pharmacological action of the drugs, and although these reactions are complex, they are not related to covalent binding [42]. There are likely some true allergic reactions caused by these and other drugs, but it is difficult to separate the pseudoallergic reactions from the true allergic reactions. It is claimed that all of these drugs also cause Stevens-Johnson Syndrome (SJS)/toxic epidermal necrolysis (TEN); SJS is a milder form of TEN. However, in the review by Mockenhaupt *et al*. while there appears to be a small risk of TEN associated with acetic acid NSAIDs, i.e. diclofenac, indomethacin, and tolmetin, there is no association with propionic acid NSAIDs, i.e. ibuprofen and naproxen [38]. Causality analysis in these cases is difficult because the rash of TEN is usually preceded by a prodromal phase, which is characterized by fever and malaise, and this prodromal phase is often treated with NSAIDs. In addition, diclofenac (discussed in detail below), and indomethacin [43] form alternative oxidative reactive metabolites. Ironically, the lack of a causal association between propionic acid NSAIDs and TEN is in contrast to oxicam-type NSAIDs and phenylbutazone, which are not carboxylic acids, but are associated with a relatively high risk of TEN [37, 38]. Drugs such as ibuprofen, which produce reactive acyl glucuronides and are classified in the warning category are really very safe except for gastrointestinal bleeding and pseudoallergic reactions, which are related to their therapeutic action [42]. The paper by Jinno *et al*., uses examples similar to the Sawamura paper, but tolmetin is listed as causing drug-induced liver injury (IDILI) [33]. However, according to the LiverTox website, “There have been no convincing cases of tolmetin induced liver injury published in the literature, and tolmetin is not mentioned as an etiologic agent in large case series of drug induced liver injury or acute liver failure.”

In the withdrawn category of the paper by Sawamura there is benoxaprofen, fenclofenac, ibufenac, and zomepirac. Benoxaprofen clearly does cause IDILI along with several other adverse reactions including photodermatitis, which implies that it is converted to a reactive species by light. In addition to forming an acyl glucuronide, it also forms an oxidative reactive metabolite [44]. In the case of zomepirac, it is not clear that it is associated with a higher risk of allergic or pseudoallergic reactions than other NSAIDs, because the risk appears to be associated with the indication for the drug [45]. In addition, zomepirac, which has a structure very similar to tolmetin, also forms an oxidative reactive metabolite [46]. As mentioned, we have little published clinical data on fenclofenac and ibufenac. Ibufenac is interesting in that it is structurally very similar to ibuprofen and the half-life of its acyl glucuronide is not markedly different. One study found that most of the covalent binding of ibuprofen and ibufenac to liver microsomes was dependent on Co-A, not the cofactor for glucuronidation, and there was significantly more covalent binding of the Co-A thioester of ibufenac than of ibuprofen [47]. In addition, the daily dose of ibufenac is up to 4 grams/day. It is certainly possible that the presumed liver injury caused by ibufenac is caused by an acyl Co-A thioester, but direct evidence is lacking. In summary, the simple correlation between acyl glucuronide stability and the risk of IDRs is much more complex than suggested by the Sawamura and Jinno papers.

A good example for the purpose of trying to differentiate various possible causal metabolites is diclofenac. Even though most papers put diclofenac and ibuprofen in the same risk category, unlike

ibuprofen, diclofenac is clearly associated with a significant risk of serious IDILI. Diclofenac forms reactive metabolites both by oxidative metabolism and acyl glucuronide formation. Initial *in vitro* studies suggested that formation of the acyl glucuronide decreases acute hepatocyte injury, but this may have no relevance for delayed onset IDILI in patients [48]. To date, the major genetic risk factor for diclofenac IDILI that has been found is the UGT2B7*2 haplotype. This suggested that the acyl glucuronide is responsible for diclofenac IDILI; however, at the time of this finding there was still a question as to whether this haplotype resulted in increased or decreased formation of the acyl glucuronide of diclofenac because the relative activity of UGT2B7*2 isoform is different for different substrates [49]. More recently, it was found the UGT2B7*2 haplotype is associated with a significant *decrease* in the formation of the diclofenac acyl glucuronide [50]. That is very strong evidence that glucuronidation of diclofenac is a protective pathway. Another possible pathway is the acyl-Co-A-ester; however, although a study by Hargus *et al*. found covalent binding of diclofenac involving the acyl glucuronide, they found no evidence of binding involving the acyl-Co-A pathway [51]. Taken together, these data strongly suggest that the oxidative pathway, rather than the glucuronidation pathway, of diclofenac is responsible for its ability to cause IDILI.

Several other carboxylic acids in the warning or withdrawn category such as furosemide, indomethacin [43], mefenamic acid [52], benoxaprofen [44], bromfenac [53], tometin, and zomepirac [46] also form reactive metabolites by an oxidative pathway. Although the data are imprecise, it is likely that these NSAIDS are associated with a much higher risk of serious IDRs than ibuprofen and naproxen even though they are placed in the same category in studies trying to correlate acyl glucuronide reactivity with IDR risk. Furosemide is interesting in that it has a furan structural alert and causes severe acute liver injury in mice [54], but even though it is classified in the "warning" group, it is a relatively safe drug with respect to IDRs. It is interesting that nearly all of the carboxylic acids associated with a high incidence of serious IDRs are NSAIDs. As mentioned above and described in the Commentary by Smith *et al*. [45], NSAIDs increase the production of leukotrienes, which are associated with pseudoallergic reactions, and the decrease in prostaglandin E, which has antiinflammatory effects. These pharmacological effects are likely to be involved in the association of NSAIDs with IDRs. As also mentioned, the non-COX-2 specific noncarboxylic acid NSAIDs are associated with a much higher risk of TEN than those that form acyl glucuronides.

Carboxylic acids are very common, not only in the structure of drugs, but also in components of our food and endogenous molecules. In addition, many drugs are metabolized to carboxylic acids that could form acyl glucuronides. In fact, there are several drugs such as enalopril that are formulated as esters to provide better oral bioavailability but require hydrolysis to a carboxylic acid to be effective. Given that many acyl glucuronides clearly can covalently bind to proteins, it is surprising that they are rarely associated with adverse reactions that are clearly due to the covalent binding of acyl glucuronides. There are several possible reasons for this. If, as proposed in Section 2, most IDRs are immune mediated and caused by reactive metabolites, there are two things that the reactive metabolite must do in order to initiate an immune response: it must produce a neoantigen, and it must cause some type of cell stress or injury leading to the release of DAMPs that activate antigen presenting cells. As mentioned in Section 2, the reactivity of the reactive metabolite appears to be very important. Although it is dangerous to infer very much from *in vitro* toxicity assays, the observation mentioned above that it was the oxidative metabolite of diclofenac that caused hepatocyte injury may provide a clue [48]. Specifically, most acyl glucuronides may simply not be able to cause cellular injury. Most of the structural alerts are functional groups that form reactive metabolites that are quite a bit more reactive, and many are also able to redox cycle; this may be important in the mechanism of IDRs. The other factor is that to invoke an immune response, the reactive metabolite must act as a hapten to form neoantigens. Clearly many acyl glucuronides covalently bind to proteins and have the potential to form neoantigens. However, in the process of activating an immune response, it is not the whole drug-modified protein that is recognized. This protein must be processed and presented in the context of HLA molecules, i.e. MHC-I or MHC-II. In the case of presentation to CD8 T cells, the presentation is in the context of MHC-I, and the length of the peptide is from 8-10 amino acids in length. In the case of presentation to CD4 T cells, the presentation is in the context of MHC-II, and the peptides are from 15 up to 24 amino acids in length. The processing of the drug-modified proteins to produce these peptides involves hydrolysis of peptide linkages, but the peptide linkage is an amide bond. Therefore, it is quite possible that protein

adducts formed from acylation of an amino group on a protein by an acyl glucuronide to form an amide bond would be removed during processing. A thioester would be even more readily cleaved during the processing of an antigen. In contrast, an adduct formed by an Amadori rearrangement would not be susceptible to such hydrolysis. However, as mentioned earlier, the inability to trap the aldehyde intermediate in the Amadori rearrangement suggests that this pathway is not usually significant with respect to covalent binding [31].

In summary, although it is plausible that reactive metabolites formed from carboxylic acids could cause IDRs, the evidence suggests that they are not a significant structural alert. In addition, even if reactive metabolites formed from carboxylic acids are sometimes responsible for IDRs, there is more than one possible mechanism by which this could occur, and simply measuring the half-life of the associated acyl glucuronide is unlikely to provide an accurate prediction of risk. Yet, pharmaceutical companies routinely study the reactivity of acyl glucuronides of drug candidates that are carboxylic acids [55].

7. CONCLUSIONS

Carboxylic acids are a very common functional group present on many molecules, including drugs. This functional group is very important for the properties of many drugs. Despite the fact that many drugs such as ibuprofen form reactive acyl glucuronides, most of these drugs are not associated with a significant risk of IDRs that are likely related to covalent binding. In most cases of drugs that have a carboxylic acid functional group and are associated with a high risk of serious IDRs, there are alternative reactive metabolites formed by oxidation. In addition, the major issue is with NSAIDs, which have pharmacological effects that confer risk. On the other hand, it is quite possible that some serious IDRs such as the liver injury associated with ibufenac are due to an acyl Co-A-thioester. It would help to have more information about the clinical characteristics of such cases and other metabolic pathways for the drug so that the association could be more accurately assessed. But if such a causal association exists, it appears to be uncommon. Association does not prove causation, especially when many of the classifications of risk are questionable. Biological systems are extremely complex, and each drug appears to have a unique pattern of biological effects, which makes any generalizations dangerous. The simple correlation that has been suggested between the reactivity of acyl glucuronides and the risk of IDRs is not at all clear. There is simply not convincing clinical evidence that the covalent binding associated with acyl glucuronide formation is responsible for serious IDRs. It is possible that acyl glucuronides can lead to IDRs, but testing their reactivity during drug development would lead to a large number of false positive and false negative results. Alternatively, acyl glucuronides may rarely or never cause IDRs. Yet, as stated earlier, many pharmaceutical companies routinely study the reactivity of acyl glucuronides of drug candidates that are carboxylic acids. In part, this is likely due to FDA guidance documents that imply that acyl glucuronides are a significant source of risk. It is possible to generate a large amount of data with high throughput *in vitro* assays; however, such data may do little to improve drug safety. What is needed is a better basic understanding of the *in vivo* immunological and other biological effects of drugs that lead to IDRs. There may be several mechanisms of IDRs. If that is the case there may never be a method that has a zero false negative predictive value, but if we understood some mechanisms well, it might be possible to have a method with a very low false positive rate. However, without a clear mechanistic understanding of the mechanisms of IDRs it is unlikely that any method will even have an acceptable false positive predictive value.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

[1] Uetrecht J. Mechanistic Studies of Idiosyncratic DILI: Clinical Implications. Front Pharmacol 2019; 10: 837. https://doi.org/10.3389/fphar.2019.00837

[2] Chung WH, Hung SI, Hong HS, *et al*. Medical genetics: a marker for Stevens-Johnson syndrome. Nature 2004; 428: 486. https://doi.org/10.1038/428486a

[3] Daly AK. Pharmacogenomics of adverse drug reactions. Genome Med 2013; 5: 5. https://doi.org/10.1186/gm409

[4] Cirulli ET, Nicoletti P, Abramson K, *et al*. A Missense Variant in PTPN22 is a Risk Factor for Drug-induced Liver Injury. Gastroenterology 2019; 156: 1707-1716.e2. https://doi.org/10.1053/j.gastro.2019.01.034

[5] Aithal GP, Ramsay L, Daly AK, *et al*. Hepatic adducts, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. Hepatology 2004; 39: 1430-1440. https://doi.org/10.1002/hep.20205

[6] Uetrecht J, Naisbitt DJ. Idiosyncratic adverse drug reactions: current concepts. Pharmacol Rev 2013; 65: 779-808. https://doi.org/10.1124/pr.113.007450

[7] Foureau DM, Walling TL, Maddukuri V, *et al*. Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced liver injury, idiopathic autoimmune and viral hepatitis. Clin Exp Immunol 2015; 180: 40-51. https://doi.org/10.1111/cei.12558

[8] Chung WH, Hung SI, Yang JY, *et al*. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. Nat Med 2008; 14: 1343-1350. https://doi.org/10.1038/nm.1884

[9] Uetrecht JP. Mechanism of drug-induced lupus. Chem Res Toxicol 1988; 1: 133-143. https://doi.org/10.1021/tx00003a001

[10] Sharma AM, Novalen M, Tanino T, Uetrecht JP. 12-OH-nevirapine sulfate, formed in the skin, is responsible for nevirapine-induced skin rash. Chem Res Toxicol 2013; 26: 817-827. https://doi.org/10.1021/tx400098z

[11] Njoku D, Laster MJ, Gong DH, Eger EI, 2nd, Reed GF, Martin JL. Biotransformation of halothane, enflurane, isoflurane, and desflurane to trifluoroacetylated liver proteins: association between protein acylation and hepatic injury. Anesth Analg 1997; 84: 173-178. https://doi.org/10.1213/00000539-199701000-00031

[12] Satoh H, Martin BM, Schulick AH, Christ DD, Kenna JG, Pohl LR. Human anti-endoplasmic reticulum antibodies in sera of patients with halothane-induced hepatitis are directed against a trifluoroacetylated carboxylesterase. Proc Nat Acad Sci USA 1989; 86: 322-326. https://doi.org/10.1073/pnas.86.1.322

[13] Klaassen CD, Fitzgerald TJ. Metabolism and biliary excretion of ethacrynic acid. J Pharmacol Exp Ther 1974; 191: 548-556.

[14] Gardner I, Zahid N, MacCrimmon D, Uetrecht JP. A comparison of the oxidation of clozapine and olanzapine to reactive metabolites and the toxicity of these metabolites to human leukocytes. Mol Pharmacol 1998; 53: 991-998.

[15] Ng W, Kennar R, Uetrecht J. Effect of clozapine and olanzapine on neutrophil kinetics: implications for drug-induced agranulocytosis. Chem Res Toxicol 2014; 27: 1104-1108. https://doi.org/10.1021/tx500183x

[16] Nakayama S, Atsumi R, Takakusa H, *et al*. A zone classification system for risk assessment of idiosyncratic drug toxicity using daily dose and covalent binding. Drug Metab Dispos 2009; 37: 1970-1977. https://doi.org/10.1124/dmd.109.027797

[17] Obach RS, Kalgutkar AS, Soglia JR, Zhao SX. Can *in vitro* metabolism-dependent covalent binding data in liver microsomes distinguish hepatotoxic from nonhepatotoxic drugs? An analysis of 18 drugs with consideration of intrinsic clearance and daily dose. Chem Res Toxicol 2008; 21: 1814-1822. https://doi.org/10.1021/tx800161s

[18] Monroe JJ, Tanis KQ, Podtelezhnikov AA, *et al*. Application of a Rat Liver Drug Bioactivation Transcriptional Response Assay Early in Drug Development that informs Chemically Reactive Metabolite formation and Potential for Drug Induced Liver Injury. Toxicol Sci 2020. https://doi.org/10.1093/toxsci/kfaa088

[19] Feng S, He X. Mechanism-based inhibition of CYP450: an indicator of drug-induced hepatotoxicity. Curr Drug Metab 2013; 14: 921-945. https://doi.org/10.2174/138920021131400114

[20] Coles B. Effects of modifying structure on electrophilic reactions with biological nucleophiles. Drug Metab Rev 1984; 15: 1307-1334. https://doi.org/10.3109/03602538409029962

[21] Gardner I, Popovic M, Zahid N, Uetrecht JP. A comparison of the covalent binding of clozapine, procainamide, and vesnarinone to human neutrophils *in vitro* and rat tissues *in vitro* and *in vivo*. Chem Res Toxicol 2005; 18: 1384-1394. https://doi.org/10.1021/tx050095o

[22] Liu ZC, Uetrecht JP. Clozapine is oxidized by activated human neutrophils to a reactive nitrenium ion that irreversibly binds to the cells. J Pharmacol Exp Ther 1995; 275: 1476-1483.

[23] Cho T, Uetrecht J. How Reactive Metabolites Induce an Immune Response That Sometimes Leads to an Idiosyncratic Drug Reaction. Chem Res Toxicol 2017; 30: 295-314. https://doi.org/10.1021/acs.chemrestox.6b00357

[24] Matzinger P. Tolerance, danger and the extended family. Annu Rev Immunol 1994; 12: 991-1045. https://doi.org/10.1146/annurev.iy.12.040194.005015

[25] Köck K, Ferslew BC, Netterberg I, *et al*. Risk factors for development of cholestatic drug-induced liver injury: inhibition of hepatic basolateral bile acid transporters multidrug resistance-associated proteins 3 and 4. Drug Metab Dispos 2014; 42: 665-674. https://doi.org/10.1124/dmd.113.054304

[26] Miller JA. Carcinogenesis by chemicals: an overview--G. H. A. Clowes memorial lecture. Cancer Res 1970; 30: 559-576.

[27] Kalgutkar AS, Dalvie D. Predicting toxicities of reactive metabolite-positive drug candidates. Annu Rev Pharmacol Toxicol 2015; 55: 35-54. https://doi.org/10.1146/annurev-pharmtox-010814-124720

[28] Spahn-Langguth H, Benet LZ. Acyl glucuronides revisited: is the glucuronidation process a toxification as well as a detoxification mechanism. Drug Metab Rev 1992; 24: 5-47. https://doi.org/10.3109/03602539208996289

[29] Bailey MJ, Dickinson RG. Acyl glucuronide reactivity in perspective: biological consequences. Chem Biol Interact 2003; 145: 117-137. https://doi.org/10.1016/S0009-2797(03)00020-6

[30] Grillo MP, Hua F. Identification of zomepirac-S-acyl-glutathione *in vitro* in incubations with rat hepatocytes and *in vivo* in rat bile. Drug Metab Dispos 2003; 31: 1429-1436. https://doi.org/10.1124/dmd.31.11.1429

[31] Gunduz M, Argikar UA, Cirello AL, Dumouchel JL. New Perspectives on Acyl Glucuronide Risk Assessment in Drug Discovery: Investigation of *In vitro* Stability, In situ Reactivity, and Bioactivation. Drug Metab Lett 2018; 12: 84-92. https://doi.org/10.2174/1872312812666180611113656

[32] Li C, Benet LZ, Grillo MP. Studies on the chemical reactivity of 2-phenylpropionic acid 1-O-acyl glucuronide and S-acyl-CoA thioester metabolites. Chem Res Toxicol 2002; 15: 1309-1317. https://doi.org/10.1021/tx020013l

[33] Jinno N, Ohashi S, Tagashira M, Kohira T, Yamada S. A simple method to evaluate reactivity of acylglucuronides optimized for early stage drug discovery. Biol Pharm Bull 2013; 36: 1509-1513. https://doi.org/10.1248/bpb.b13-00329

[34] Sawamura R, Okudaira N, Watanabe K, *et al*. Predictability of idiosyncratic drug toxicity risk for carboxylic acid-containing drugs based on the chemical stability of acyl glucuronide. Drug Metab Dispos 2010; 38: 1857-1864. https://doi.org/10.1124/dmd.110.034173

[35] Uetrecht JP, Trager W. Drug Metabolism: Chemical and Enzymatic Aspects. New York: Ihforma Health Care; 2007. https://doi.org/10.1201/b14488

[36] Mittmann N, Knowles SR, Gomez M, Fish JS, Cartotto R, Shear NH. Evaluation of the extent of under-reporting of serious adverse drug reactions: the case of toxic epidermal necrolysis. Drug Saf 2004; 27: 477-487. https://doi.org/10.2165/00002018-200427070-00004

[37] Guillaume JC, Roujeau JC, Revuz J, Penso D, Touraine R. The culprit drugs in 87 cases of toxic epidermal necrolysis (Lyell's syndrome). Arch Dermatol 1987; 123: 1166-1170. https://doi.org/10.1001/archderm.1987.01660330077013

[38] Mockenhaupt M, Viboud C, Dunant A, *et al*. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. J Invest Dermatol 2008; 128: 35-44. https://doi.org/10.1038/sj.jid.5701033

[39] Roujeau JC, Kelly JP, Naldi L, *et al*. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. N Engl J Med 1995; 333: 1600-1607. https://doi.org/10.1056/NEJM199512143332404

[40] Rodriguez J. Thrombocytopenia associated with meclofenamate. Drug Intell Clin Pharm 1981; 15: 999. https://doi.org/10.1177/106002808101501217

[41] Woo SR, Corrales L, Gajewski TF. Innate immune recognition of cancer. Annu Rev Immunol 2015; 33: 445-474. https://doi.org/10.1146/annurev-immunol-032414-112043

[42] Gómez F, Perkins JR, García-Martín E, Canto G, Cornejo-García JA. Genetic basis of hypersensitivity reactions to nonsteroidal anti-inflammatory drugs. Curr Opin Allergy Clin Immunol 2015; 15: 285-293. https://doi.org/10.1097/ACI.0000000000000178

[43] Ju C, Uetrecht JP. Oxidation of a metabolite of indomethacin (Desmethyldeschlorobenzoylindomethacin) to reactive intermediates by activated neutrophils, hypochlorous acid, and the myeloperoxidase system. Drug Metab Dispos 1998; 26: 676-680.

[44] Mitchell M, Lietman P. Evidence for the role of reactive metabolites in hepatoxicity of benoxaprofen and other nonsteroidal anti-inflammatory drugs (NSAIDS). Hepatology 1983; 3: 808.

[45] Smith DA, Hammond T, Baillie TA. Safety Assessment of Acyl Glucuronides-A Simplified Paradigm. Drug Metab Dispos 2018; 46: 908-912. https://doi.org/10.1124/dmd.118.080515

[46] Chen Q, Doss GA, Tung EC, *et al*. 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 2006; 34: 145-151. https://doi.org/10.1124/dmd.105.004341

[47] Darnell M, Breitholtz K, Isin EM, Jurva U, Weidolf L. Significantly Different Covalent Binding of Oxidative Metabolites, Acyl Glucuronides, and S-Acyl CoA Conjugates Formed from Xenobiotic Carboxylic Acids in Human Liver Microsomes. Chem Res Toxicol 2015; 28: 886-896. https://doi.org/10.1021/tx500514z

[48] Kretz-Rommel A, Boelsterli UA. Diclofenac covalent protein binding is dependent on acyl glucuronide formation and is inversely related to P450-mediated acute cell injury in cultured rat hepatocytes. Toxicol Appl Pharmacol 1993; 120: 155-161. https://doi.org/10.1006/taap.1993.1097

[49] Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. Gastroenterology 2007; 132: 272-281. https://doi.org/10.1053/j.gastro.2006.11.023

[50] Lazarska KE, Dekker SJ, Vermeulen NPE, Commandeur JNM. Effect of UGT2B7*2 and CYP2C8*4 polymorphisms on diclofenac metabolism. Toxicol Lett 2018; 284: 70-78. https://doi.org/10.1016/j.toxlet.2017.11.038

[51] Hargus SJ, Amouzedeh HR, Pumford NR, Myers TG, McCoy SC, Pohl LR. Metabolic activation and immunochemical localization of liver protein adducts of the nonsteroidal anti-inflammatory drug diclofenac. Chem Res Toxicol 1994; 7: 575-582. https://doi.org/10.1021/tx00040a014

[52] Venkataraman H, den Braver MW, Vermeulen NP, Commandeur JN. Cytochrome P450-mediated bioactivation of mefenamic acid to quinoneimine intermediates and inactivation by human glutathione S-transferases. Chem Res Toxicol 2014; 27: 2071-2081. https://doi.org/10.1021/tx500288b

[53] Driscoll JP, Yadav AS, Shah NR. Role of Glucuronidation and P450 Oxidation in the Bioactivation of Bromfenac. Chem Res Toxicol 2018; 31: 223-230. https://doi.org/10.1021/acs.chemrestox.7b00293

[54] Mitchell JR, Snodgrass WR, Gillette JR. The role of biotransformation in chemical-induced liver injury. Environ Health Perspect 1976; 15: 27-38. https://doi.org/10.1289/ehp.761527

[55] Walles M, Brown AP, Zimmerlin A, End P. New Perspectives on Drug-Induced Liver Injury Risk Assessment of Acyl Glucuronides. Chem Res Toxicol 2020; 33: 1551-1560. https://doi.org/10.1021/acs.chemrestox.0c00131

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