Review

Assessment of nonsteroidal anti-inflammatory drug-induced hepatotoxicity

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Introduction: Liver toxicity related to NSAIDs is of outstanding importance because of the wide use of these drugs. NSAIDs are responsible for roughly 10% of the total of cases of drug-induced hepatotoxicity. The assessment of NSAID-induced hepatotoxicity, presently based on clinical and analytical biomarkers, is critical for early diagnosis and immediate withdrawal of the causing drug.

Areas covered: The review presents an overview of current knowledge of the assessments of NSAID-induced hepatotoxicity with emphasis on the causative drugs, the NSAID-specific mechanisms involved, and a summary of genetic and non-genetic risk factors. Additionally, the authors discuss genetic factors which show NSAID-specific risk, namely CYP2C, UGT2B7, GSTM1 and GSTT1, as well as HLA alleles. The paper includes a list of the NSAID 'usual suspects' that cause hepatotoxicity based on the integrated information of drug-induced hepatotoxicity databases.

Expert opinion: The ultimate goal of this research is pre-prescription testing. Unfortunately, genetic testing, alone, is not sufficient to predict NSAID-induced hepatotoxicity. The development of genetic biomarkers capable of identifying at-risk individuals will not be complete until we develop the ability to fully characterize patients’ phenomes and the phenome-genome interaction in patients with NSAID-induced hepatotoxicity. Additionally, a characterization of the metabolic profile of the causative drug in patients with NSAID-induced hepatotoxicity would add crucial information which is presently disregarded in most studies. The full development of robust biomarkers will require the combination of several disciplines including causal statistics, phenomics, genomics, transcriptomics and metabonomics.

Keywords: bioactivation, genetic markers, hepatotoxicity, metabolism, NSAIDs

Expert Opin. Drug Metab. Toxicol. (2011) 7(7):817-828

1. Introduction

NSAIDs are among the most widely used drugs both as prescription and over-the-counter drugs. Six percent of the adult population in the US report the use of at least one prescription of NSAID a month [1]. Over 30 million people receive NSAIDs daily [2] and nearly 25% of the population have experienced NSAID-related side effects that require medical care [3]. Several studies and databases have reported patients who developed fatal hepatic toxicity related to NSAID use [4-7]. Although it is generally accepted that liver toxicity is a relatively rare adverse reaction to NSAIDs [4,8], liver toxicity related to NSAIDs is of outstanding importance because of the wide use of these drugs. In fact, NSAIDs are responsible for roughly 10% of the total of cases of drug-induced hepatotoxicity [8-15].
initially spontaneous reports of NSAID-related hepatotoxicity signaled a potential problem. Then, systematic reviews indicated that NSAID-related hepatotoxicity is not a rare event and that the incidence of NSAID-related liver injuries resulting in hospitalization ranged from 3.1 to 23.4/100,000 patient-years [4]. Moreover, about one out of four patients who develop jaundice die as a result of NSAID-induced liver failure [14]. A comprehensive assessment of NSAID-induced hepatotoxicity, based on clinical and analytical biomarkers, is critical for early diagnosis and immediate withdrawal of the causing drug. In this invited review, we analyze relevant factors in the assessment of NSAID-induced hepatotoxicity and potential biomarkers which may be useful in the prediction of serious clinical outcomes and may be used in pre-prescription testing and primary prevention of this potentially fatal adverse hepatic reaction.

2. NSAID-induced hepatotoxicity assessment

The diagnosis of NSAID-related liver hepatotoxicity is not an easy task. It includes the identification of a temporal relationship between the use of the drug and the clinical event, although the time to onset is typically delayed by weeks or months in idiosyncratic drug-induced liver injury [16]. Other factors relevant for diagnosis are the type of the drug used (that is, whether the drug has been related to liver hepatotoxicity in spontaneous reports or in published databases [9]), the biochemical pattern, the presence of known risk factors and the exclusion of alternative causes. Unfortunately, mild cases are underreported and it is likely that many of these mild hepatotoxicity events related to NSAID use are unnoticed by the scientific community.

It should be stated that the frequency of NSAID-related hepatotoxicity is too low to assess accurately in clinical trials [17]. Spontaneous reports do not allow the determination of incidence or relative risk, and available databases are scarce and based on heterogeneous criteria. An attempt to integrate diverse databases has resulted in a unified list based on international collaborative work. This list of drugs includes several NSAIDs, which constitute a prominent pharmacological group in drug-induced hepatotoxicity, although differences in the association of NSAIDs across databases are observed [9]. Factors that have been proposed to influence the risk of developing drug-induced liver injury include age, gender, chronic alcohol consumption, concomitant drugs, underlying disease states, obesity, diabetes mellitus type 2 and insulin resistance [18]. However, when age and gender were analyzed in a series of 66 patients with NSAID-induced hepatotoxicity, none of these factors significantly influenced the risk although older age was a predictor of cholestatic expression of drug-induced hepatotoxicity while younger age was related to hepatocellular pattern [19]. Known risk factors for drug-induced hepatotoxicity may be, at least in part, drug-specific. Risk factors for drug-induced hepatotoxicity have been recently reviewed and are not discussed here in detail because in most cases the associations were identified in patients with drug-induced hepatotoxicity related to drugs other than NSAIDs [16,19]. It is worthy of mention, however, that NSAID-related hepatotoxicity is notably more frequent in patients with concomitant medication with other potentially hepatotoxic drugs [20].

Regarding the type of the drug, Table 1 summarises the NSAIDs and other non-NSAID analgesics of common use which caused hepatotoxicity in the unified list including three major registries in Spain, Sweden and the US, as well as other data sources [4,9,13,21]. Besides the non-NSAID analgesic acetaminophen, which is the commonest cause of intrinsic hepatotoxicity, seven NSAIDs account for the vast majority of NSAID-induced hepatotoxicity. These seven NSAIDs together make up about 99% of all the cases related to NSAID use. According to their chemical structures, these drugs belong to five groups, namely, from higher to lower frequency: acetic acid derivatives (46.5%), propionic acid derivatives (25.7%), salicylates (12%), enolic acid derivatives (9.3%) and sulfonanilides (5.8%). The average of patients with NSAID-induced hepatotoxicity who develops acute liver failure (ALF) is 6%. The highest risk of developing NSAID-induced ALF is related to the use of ibuprofen (9.4%), aspirin (8.2%), naproxen (8.1%) and nimesulide (6.6%) whereas lower risk is observed with diclofenac (5.1%), piroxicam (4.1%) and sulindac (2.5%). Whereas most NSAID-induced hepatotoxicity cases are related to individual susceptibility, aspirin is a dose-related hepatotoxin [22], thus, suggesting that the mechanisms underlying NSAID-induced hepatotoxicity are not identical for all NSAIDs. Figure 1 summarizes the structure of the...
NSAIDs and analgesics identified as a major source of drug-induced hepatotoxicity. The chemical structures of the NSAIDs involved in hepatotoxicity are heterogeneous and, therefore, the chemical group is a poor index for assessing the risk. For instance, within the group of acetic acid derivatives virtually all the risk is related to only two drugs (diclofenac and sulindac) whereas the rest of the NSAIDs in this chemical group are related with an extremely low frequency (aceclofenac, acemetacin, indomethacin or ketorolac) or no cases at all have been reported. This example is valid for all chemical groups of NSAIDs, and in most chemical groups the vast majority of cases are related to one or two drugs (Table 1). It is of note that, with regard to drug-induced hepatotoxicity, selective COX-2 inhibitors and fenamic acid derivatives are relatively safe, as compared to other chemical groups of NSAIDs. Besides the data shown in Table 1, isolated cases of hepatotoxicity have been attributed to other NSAIDs such as diclofenac, ibuprofen or aspirin among those NSAIDs commonly related to hepatotoxicity is not surprising. Ideally, the evaluation of the hepatotoxic potential of a particular NSAID should be adjusted to figures of drug prescription that allow an approximation to population’s exposure to a particular drug. This is a major cause of heterogeneity in studies on the frequency of association of a particular NSAID with hepatotoxicity which were carried out in diverse countries [13,30]. A good example of this heterogeneity is nimesulide. In 2002, Finland and Spain suspended the marketing of nimesulide because it was associated with a high frequency of hepatotoxicity [21,31]. Today, nimesulide has been withdrawn in Europe, but after the discontinuation of use in Spain and Finland, nimesulide continued to be marketed in several countries and for many years it was the most prescribed NSAID in Italy and in Portugal. In this regard, it should be stated that in the years in which nimesulide was

Table 1. NSAIDs and analgesic drugs in common use which cause hepatotoxicity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Generic name</th>
<th>Percentage of total cases of liver injury (NSAID + analgesics)</th>
<th>Percentage of total cases of liver injury (NSAIDs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylates</td>
<td>Aspirin</td>
<td>6.9%</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Salsalate</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>Acetic acid derivatives</td>
<td>Aceclofenac</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Acemetacin</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Diclofenac</td>
<td>19.5%</td>
<td>34.1%</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Ketorolac</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
<td>7.1%</td>
<td>12.4%</td>
</tr>
<tr>
<td>Propionic acid derivatives</td>
<td>Dextroprofen</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>8.4%</td>
<td>14.6%</td>
</tr>
<tr>
<td></td>
<td>Ketoprofen</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>6.4%</td>
<td>11.1%</td>
</tr>
<tr>
<td></td>
<td>Oxaprozin</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>Enolic acid derivatives</td>
<td>Droxicam</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Meloxicam</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Piroxicam</td>
<td>5.4%</td>
<td>9.3%</td>
</tr>
<tr>
<td></td>
<td>Tenoxicam</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>Selective COX-2 inhibitors</td>
<td>Celecoxib</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Rufecoxib</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td></td>
<td>Valdecoxib</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>Sulfonanilides</td>
<td>Nimesulide</td>
<td>3.3%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Non-NSAID analgesics</td>
<td>Acetaminophen</td>
<td>42.7%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Methamizole</td>
<td>&lt; 0.1%</td>
<td>-</td>
</tr>
</tbody>
</table>

Frequencies are based on the WHO Program for International Drug Monitoring, Vigibase™ and on drug-induced liver injury registries from Europe and the US, as well as other published data sources [4,9,13,21]. Liver injury cases were patients with clinically significant liver injury according to standard diagnostic criteria (see the text for details). Total cases (100%) for NSAID + analgesics = 10,506. Total cases for NSAIDs = 6023.
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marketed in Spain it has an average use of defined daily dose (DDD) per 1000 inhabitants and day of 0.586, was by far higher than those of aspirin (0.175) or sulindac (0.035) (data from the Agencia Española de Medicamentos y Productos Sanitarios, Spanish Ministry of Health; available on the website in [32]). Therefore, it could be hypothesized that a wide use would have influenced the relatively high frequency of nimesulide-induced hepatotoxicity observed in these years. Because in Spain NSAIDs, as well as all other drugs, are marketed in pharmacies only, the control of NSAID consumption by the health system is rigorous. This allowed us to evaluate the risk of NSAID-induced hepatotoxicity by adjusting the number of cases diagnosed within the years 1996 – 2008 to the average use of these drugs within the years 1994 – 2006, expressed in DDDs, and to the number of years which every drug was marketed. Figure 2 shows the crude frequencies and the adjusted frequencies of NSAID-induced hepatotoxicity presently registered in the Spanish database, available in the website in [33]. We only included NSAIDs which caused two or more events of hepatotoxicity. Crude frequencies indicate that ibuprofen and diclofenac are the main NSAIDs responsible for hepatotoxicity in the registry. However, when frequencies are adjusted by DDD and the number of years in the market, nimesulide and aspirin show the strongest association with hepatotoxic events. This confirms the hypothesis for a high association of nimesulide with hepatotoxicity in Spain, even if drug use is taken into consideration. As stated previously, one of the factors used in the assessment of NSAID-induced hepatotoxicity is whether the drug has been related to liver hepatotoxicity in spontaneous reports or in published databases, that is, whether the drug belongs to the list of ‘usual suspects’ of hepatotoxicity. In this regard, the list of NSAIDs shown in Table 1 and Figure 2 may be of help in the assessment of NSAID-induced hepatotoxicity.

Another parameter used in assessment of hepatotoxicity is the morphological pattern of liver injury. Ideally, liver histology is the best tool for defining the pattern of liver damage, although it is not diagnostic and can be considered at best compatible with NSAID-induced hepatotoxicity. However, a liver biopsy specimen is often not available, and in the absence of histological data the pattern of drug-related liver injury is classified according to laboratory data [34]. The classification scheme was proposed by the Council for International Organizations of Medical Sciences and updated by the FDA Drug Hepatotoxicity Committee and recently by the Phenotyping Standardization Project Group (The international Severe Adverse Events Consortium’s Phenotyping Standardization Project) [35]. The pattern of serum enzymes elevations defines whether the hepatic injury is ‘hepatocellular’, ‘mixed’ or ‘cholestatic’, which are defined
by calculation of the ‘R value’. The R value is calculated by dividing the alanine aminotransferase by the alkaline phosphatase using multiples of the upper limit of the normal range for both values. Calculation of the type of liver damage according to this rule correlates quite well with the underlying histological lesion and has proven prognostic value [16]. According to this classification, most NSAIDs are related to hepatocellular damage although some are related to cholestatic or mixed damage. The acute hepatocellular (cytotoxic, cytolytic) type of liver injury is the most common expression of hepatotoxicity, especially in younger individuals [10,12,19,36,37] and it is observed with many drugs. In patients with acute hepatocellular drug-induced hepatitis, the presence of jaundice is the most significant predictor of mortality/liver transplantation. The observation by Hyman Zimmerman, known as ‘Hy’s rule’ [38] predicts a mean mortality (or its surrogate marker, liver transplantation) of 10% (range 5 – 50%) for jaundiced patients with acute toxic hepatocellular damage. The NSAIDs which cause hepatocellular damage include aceclofenac, diclofenac, drotrecogin, ibuprofen, indomethacin, ketoprofen, ketorolac, meloxicam, nimesulide, piroxicam, rofecoxib and sulindac. Drug-induced acute cholestatic and mixed lesions are less severe in the short-term outcome than the hepatocellular type, but the resolution of cholestatic and mixed lesions are generally slower, with a higher likelihood towards chronicity [39]. Cholestatic hepatotoxicity is caused by many drugs, including NSAIDs, which characteristically produce liver injury with minimal or absent accompanying inflammation, presumably by interfering with bilirubin and bile acids export from the canaliculi. The NSAIDs which cause hepatotoxicity with a cholestatic pattern include celecoxib, drotrecogin, ibuprofen, naproxen, piroxicam, rofecoxib and sulindac. In mixed hepatic injury, the clinical and biological picture is intermediate between the hepatocellular and the cholestatic patterns, and features of either type may predominate. Almost all drugs that produce cholestatic injury are also capable of inducing a mixed pattern. NSAIDs which cause mixed hepatotoxicity include drotrecogin, ibuprofen, piroxicam, rofecoxib and sulindac. It is interesting to note that the ‘signature’ (consistent clinical, pathologic and latency presentation) for a given NSAID may be variable among individuals. Underlying mechanisms of damage are still unknown but there seem to be a cross-talk between drug metabolism and the immune system. Recently, much attention has been dedicated to the study of genetic factors related to host susceptibility to drug-induced hepatotoxicity. Most of these studies have focused on the identification of common variants that confer a small risk of hepatotoxicity and are based both on genome-wide association studies (GWAS) [27] and, mainly, on candidate gene association studies (CGAS). Many of the susceptibility genes analyzed are related to the mechanisms proposed for drug-induced hepatotoxicity, which are the following: i) reactive metabolite hypothesis, ii) immune-mediated hepatotoxicity, iii) ‘danger
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signal hypothesis and iv) alterations in mitochondrial function. These mechanisms have recently been revised [16] and are not described in detail. An interesting mechanistic classification for genetic factors related to drug-induced hepatotoxicity was that formulated by Kaplowitz, which classified the events leading to drug-induced hepatotoxicity as ‘upstream’, that is, drug-specific mechanisms involved in the initial injury, and ‘downstream’, which are general mechanisms that balance injurious versus protective cellular pathways [40]. This model was implemented in an elegant model with three consecutive steps [41]. The first step is initial injury, caused by the parent drug or more frequently by its reactive metabolites, the second step is mitochondrial permeability transition and the third step is hepatocyte death [41,42]. The first step is drug-specific because in this step the drug or their metabolites cause cell stress, mitochondrial impairment or specific immune reactions. Further steps are not drug-specific as these involve general mechanisms of mitochondrial function and apoptosis.

Because the present review focuses on the assessment of NSAID-induced hepatotoxicity, only drug-specific mechanisms related to NSAIDs and general ‘downstream’ mechanisms which are not drug-specific are discussed here. Further information on genes related to non-NSAID drugs can be accessed in recent reviews on pharmacogenetics and pharmacogenomics in drug-induced hepatotoxicity [16,42,43].

With regard to drug-specific mechanisms, drug biotransformation is believed to be a relevant process in NSAID-induced hepatotoxicity. Most NSAIDs are extensively metabolized in humans and it has been shown that the activity of NSAID-metabolizing enzymes shows significant interindividual and interethnic variation [44]. Genetic and environmental factors are responsible for this variability. It has been recently shown that drugs with a high degree of hepatic metabolism, and particularly that mediated through cytochrome P450 (CYP) CYP2C enzymes, are more likely to cause hepatotoxicity than those with a low metabolism [45]. Most enzymes that metabolize NSAIDs are polymorphic. Variant genes cause abolished, reduced, altered or increased enzyme activity because of complete gene deletions, single nucleotide polymorphisms that occur isolated or combined, and gene duplications. Individuals carrying enzyme-inactivating polymorphisms display higher drug plasma concentrations and lower clearance rates when treated at standard doses. Therefore, an increased prevalence and severity of adverse drug reactions, including hepatotoxicity, could be expected among subjects carrying enzyme-inactivating mutations, when receiving NSAIDs that are substrates of the defect enzyme.

Although NSAIDs causing hepatotoxicity constitute a chemically heterogeneous group of drugs that differ in their therapeutic efficacy and toxicity (Figure 1), two enzymes, namely CYP2C8 and CYP2C9, are the major enzymes involved in the first steps of the metabolism or participate in secondary metabolic steps of most NSAIDs shown in Table 1 [44] with few exceptions. Regarding aspirin, the hepatotoxic salicylic acid is partly metabolized by CYP enzymes, mainly CYP2E1 and CYP3A4, although controversial information suggest a participation of CYP2C9, together with the xenobiotic/medium chain fatty acid:CoA ligase (ACSM2), and UDP-glucuronosyltransferase enzymes [46,47] and, therefore, CYP enzymes are likely to play a role, though secondary, in the bioactivation of aspirin metabolites. Besides the putative implications of CYP2C enzymes in other diseases [44,48,52], it has been shown that genetic variations in CYP2C enzymes cause variations in NSAID metabolism. CYP2C8 has a partial overlapping in substrate specificity with CYP2C9, and growing evidence indicates that NSAIDs initially thought to be CYP2C9 substrates are also metabolized by CYP2C8 [53,54]. The CYP2C8*3 variant allele causes impaired biodisposition of ibuprofen [54]. Other variant CYP2C8 alleles are CYP2C8*2, which is related to increased $K_{m}$ of the enzyme [55], CYP2C8*4, which causes a marginal decrease in the metabolic capacity, and CYP2C8*5, which causes a frame shift and an early stop codon [56]. The most common CYP2C8 variant allele in caucasian subjects, designated as CYP2C8*3, is in linkage disequilibrium with CYP2C9*2. This linkage is disrupted in some pathologies [57], but is consistently observed in healthy subjects. The presence of CYP2C8*3 plus CYP2C9*2 variant alleles has been associated with decreased metabolism of ibuprofen [54,58]. The presence of variations in the CYP2C9 gene is related to impairment in the metabolism of several NSAIDs [44]. Moreover, the enzyme activity can be modified by concomitant therapy; for instance, CYP2C8 can be inhibited by trimethoprim [59] and induced by paclitaxel, a CYP2C8 substrate [60,61]. Therefore, it is unlikely that the analysis of single nucleotide polymorphisms alone would give an absolutely true picture of the CYP2C metabolic capacity in vivo. Based on the hypothesis of genetically-determined susceptibility to NSAID-induced hepatotoxicity linked to drug biotransformation, various studies CGAS were conducted to test whether CYP2C8 and/or CYP2C9 polymorphisms can be considered as risk factors for NSAID-induced hepatotoxicity [62,63].

Aithal et al. investigated the influence of CYP2C9*2 and CYP2C9*3 in hepatotoxicity caused by diclofenac in 24 patients, with negative findings [62]. Another study by Pachkoria et al. had a similar sample size and did not identify a positive association [63]. Daly et al. analyzed the polymorphisms of several enzymes involved in the metabolism of diclofenac with relation to the risk of developing hepatotoxicity and found a positive association for CYP2C8 variant alleles, although the association was marginal [8]. One case report indicated the presence of homozygous CYP2C9*3 genotype associated with severe hepatotoxicity during the use of the disease-modifying antirheumatic drug leflunomide [64]. CYP2E1 is the major enzyme involved in the production of hepatotoxic metabolites from general anesthetics [65] and has been related to the production of the hepatotoxic acetyaminophen metabolite N-acetyl-p-benzquinone imine [66]. In agreement with the hypothesis of CYP2E1-mediated
bioactivation of acetaminophen is the fact that deletion of CYP2E1 and CYP1A2 prevented paracetamol toxicity in a mouse line [67]. Among the 13 variant CYP2E1 alleles described so far, the most common is the variant CYP2E1*5 allele, initially designated as C2 allele, which is located in the 5' flanking region [68]. Another variant allele, designated as CYP2E1*2, causes a substitution of the amino acid 76, and is related with reduced enzyme activity in vitro [69], although it has recently been shown that CYP2E1*2 is an extremely rare allelic variant [70]. Because CYP2E1 allelic variants related to altered enzyme activity are rare, no CGAS studies on the effect of paracetamol-induced hepatotoxicity have been carried out, and evidence of a link between CYP2E1 genetic variants and drug-induced hepatotoxicity are limited to anti-tuberculosis drugs (for a recent meta-analysis see [71]). Other CYP enzymes related to drug-induced hepatotoxicity are CYP2D6, CYP3A4 and CYP3A5. However, the association of variations in these genes with drug-induced hepatotoxicity is related to non-NSAID drugs. Because no cases of NSAID-induced hepatotoxicity have been reported to be associated with variations in these CYP genes, these will not be detailed here. For recent reviews, see [16,42].

With regard to other NSAID-metabolizing enzymes related to hepatotoxicity, uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7) was investigated with regard to diclofenac hepatotoxicity. Diclofenac metabolism is carried out by diverse hydroxylation pathways and by acyl glucuronidation. About 50% of diclofenac is eliminated as 4-hydroxydiclofenac, a CYP2C9 product [16]. Daly et al. [8] found that the variant UGT2B7*2 allele is more common in patients with hepatotoxicity caused by diclofenac, compared with healthy controls and with patients receiving diclofenac without developing hepatotoxicity. With regard to glutathione S-transferases (GSTs), besides their relevance in cancer risk and in the risk of ethanol-induced liver damage [72-73], these enzymes are likely to play a double role on defense against NSAID-induced hepatotoxicity, because GSTs metabolize reactive drug metabolites and GSTs play a general ‘downstream’ protection mechanism against drug-induced hepatotoxicity. In a CGAS study involving 154 cases of drug-induced hepatotoxicity, the presence of the double-null genotype for GSTTI and GSTM1 is associated with a 2.7-fold increased risk of developing drug-induced hepatotoxicity regardless of the type of drug involved, thus suggesting a general drug-unspecific mechanism [74]. Other studies on the role of GST and drug-induced hepatotoxicity support this general role [75-78]. Interestingly, in the Spanish study the association of the genetic risk with NSAID-induced hepatotoxicity was strikingly high with an 8.8-fold increased risk for carriers of the double-null genotype [74]. This indicates that besides the general ‘downstream’ effect, GSTs polymorphisms are likely to play a drug-specific ‘upstream’ role in NSAID-induced hepatotoxicity and points out that common genetic variations may contribute to susceptibility of developing hepatotoxicity with multiple drugs.

The role of the immune system in the susceptibility to drug-induced hepatotoxicity is emerging from GWAS studies. The HLA-DRB1*1501-DQB1*0602-DRB5*0101-DQA1* 0102 haplotype has been recently found a risk factor for the selective NSAID COX-2 inhibitor lumiracoxib [27]. If the mechanism involved in the association of the haplotype and the risk is, as proposed by the authors, an immune reaction triggered by lumiracoxib or by adducts formed with reactive lumiracoxib metabolites, it is likely that this mechanism may be relevant to other NSAIDs. However, the same haplotype was associated with amoxicillin and clavulanate-induced hepatotoxicity [79], two drugs which have no structural similarity with lumiracoxib, thus suggesting that the mechanism underlying the association of the haplotype risk and hepatotoxicity is not drug-specific. Further studies aimed to analyze whether the risk haplotype is related to hepatotoxicity caused by NSAIDs other than lumiracoxib, particularly other coxibs, are urgently required to have a better understanding of such interesting association. Diclofenac-induced hepatotoxicity in a northern European population has been related to polymorphisms in genes coding for cytokines (IL-4 and -10) and to ATP-binding cassette transporters (ABCC2) although these findings could not be replicated in a Spanish drug-induced hepatotoxicity cohort [42,80,81]. Interestingly, IL-10 polymorphisms have been also related to the risk of advanced chronic alcoholic liver disease thus suggesting similarities between alcoholic and drug-induced hepatotoxicity [82]. For recent reviews on general mechanisms involved in the pharmacogenomics of drug-induced hepatotoxicity, see [16,42,43].

Particular attention should be paid to the mechanisms involving mitochondrial oxidative stress management in NSAID-induced hepatotoxicity. Because most NSAIDs are metabolized by Phase I enzymes, these drugs or their major metabolites produce reactive species [44]. Molecular damage from reactive species is important in the pathogenesis of toxic liver injury, as has been recently demonstrated with the NSAID sulindac [83]. The mitochondrial enzyme manganese superoxide dismutase (MnSOD, SOD2) is the major scavenger of mitochondrial superoxide. It has been shown that SOD2 knockout mice have increased susceptibility to hepatotoxicity caused by the NSAID nimesulide [84]. In addition to SOD2, glutathione peroxidases can modulate the intracellular level of hydrogen peroxide. Glutathione peroxidase 1 (GPX1) is part of the cellular antioxidant defense system by catalyzing the reduction of hydrogen peroxide and various organic hydroperoxides using reduced glutathione as a co-substrate. GPX1 is the main glutathione peroxidase in the mammalian liver and plays a significant role in preventing mitochondrial oxidative stress.

Two independent CGAS studies have revealed that the MnSOD mutant C allele is associated with drug-induced hepatotoxicity. This mutant allele encodes a protein with the amino-acid substitution Val16Ala (rs4880). In the first study NSAIDs were not independently analyzed and, therefore, only a general association with hepatotoxic drugs
could be detected, with a 2.4-fold increased risk for carriers of the variant allele [76]. Another study analyzed the same polymorphism and found a 2.3-fold increased risk for homozygous carriers of the variant allele [85]. The risk of developing hepatotoxicity associated with the SOD2 polymorphism was higher in patients with cholestatic/mixed hepatotoxicity, and with patients taking drugs that are mitochondria hazardous or that produce reactive metabolites, but no particularly high risk was observed among patients with NSAID-induced hepatotoxicity [85]. The same study analyzed two GPX1 and one GPX4 polymorphisms. The GPX1 polymorphism causing the amino-acid substitution Pro200Leu (rs1050450) was associated with a 5.1-fold increased risk for cholestatic hepatic damage. Moreover, when risk was analyzed with regard to the combination of GPX1 polymorphism and GST null alleles, a cumulative effect on hepatotoxicity was observed. When cases of NSAID-induced hepatotoxicity are separately analyzed, the increased risk related to the presence of the GPX1 Leu allele is 2.3-fold, although the statistical significance was not conclusive (p = 0.065) due to the small size of patients with NSAID-induced hepatotoxicity (n = 21). Taken together, these findings support a genetic basis for susceptibility to drug-induced hepatotoxicity related to mitochondrial damage, but do not suggest that this is due to a NSAID-specific mechanism [85]. Recently, it has been described that an association of the keratin variants KRT8 and KRT18 predisposed to ALF among patients with acetaminophen-induced hepatotoxicity [86]. Whether these genetic associations are specifically related to NSAID-induced hepatotoxicity remains to be elucidated. Diminished expression of the hepatocellular transporter ABCB11 (BSEP), which mediates the elimination of bile salts from hepatocytes into the bile canaliculi, might influence the level of cellular exposure to reactive metabolites and cytotoxic bile salts. Patients with NSAID-induced hepatotoxicity who are homozygous for the C allele (ABCB11 1331T > C polymorphism) are at increased risk of developing liver toxicity with a hepatocellular pattern (odds ratio = 2.9; 95% CI 1.6 - 5.4; P corrected for multiple comparison (Pc) > 0.002), as a manifestation of accumulation of toxic bile salts in hepatocytes. This study suggests that the BSEP transporter might represent a novel pharmacological site of interaction for NSAIDs [87].

All these gene variations, although not directly associated with NSAIDs, should be taken into consideration in the assessment of NSAID-induced hepatotoxicity because these genes influence general mechanisms that may modify the development or the clinical presentation and outcome of hepatotoxicity.

3. Conclusion

Clinical and analytical assessment of NSAID-induced hepatotoxicity is crucial for early diagnosis and immediate withdrawal of the causing drug. In the absence of robust biomarkers, the diagnosis is based on a temporal relationship, clinical and biochemical parameters and estimation of probabilities with a list of NSAIDs which are 'usual suspects' for drug-induced hepatotoxicity. Genetic association studies are promising for producing biomarkers, but much additional work is required before we can develop pre-medicating testing and primary prevention based in biomarkers.

4. Expert opinion

Besides a rapid and correct diagnosis, genetic and non-genetic risk factors should be considered in the assessment of NSAID-induced hepatotoxicity. Milestone findings in this field are the development of a rule for the evaluation of the morphological pattern of the liver damage without biopsies [16,34], the detailed evaluation of the non-genetic risk factors which may help in the diagnosis [88], elaboration of a list of causative drugs, which are of great help in the estimation of a causality -effect probability [90], elaboration of mechanistic models of the disease [40,41] and identification and characterization of genetic risk factors in association studies. Major weaknesses include the fact that mild cases are often underreported, and an even more important is the fact that variability in diagnosis, therapy and clinical evaluation greatly differs across different countries. This makes it difficult to obtain consistent associations between risk factors and NSAID-induced hepatotoxicity. In our opinion, another major weakness in this field is related to phenomics; our ability to characterize the full set of phenotypes of an individual (phenome) lags behind our ability to characterize genomes [88], and this hampers the development of robust biomarkers.

The ultimate goal in NSAID-induced hepatotoxicity research should include the development of biomarkers for pre-medicating testing and primary prevention. However genetic testing alone, although promising, is not sufficient to predict NSAID hepatotoxicity. The analysis of non-genetic risk factors [18] (age, gender, daily dose, the metabolic profile of the NSAID, drug interactions, alcohol intake and co-morbidities) should be combined in the assessment with the analysis of genetic factors (genetic polymorphisms in Phase I and II enzymes, drug transporters, HLA class antigens, cytokines or genes related to mitochondrial function). A crucial point that should be taken into consideration is that genetic associations discovered in one population are not often replicated in another population. A study comparing the French and the Spanish databases on NSAID-induced hepatotoxicity revealed that some NSAIDs showed different risk levels in the two countries. These differences were attributed to differences in drugs use and genetic or environmental factors [30]. A potential cause for these discrepancies is the occurrence of interethnic and intraethnic variability in polymorphisms of drug-metabolizing enzymes such as CYP2C, GSTs enzymes and, to a lesser degree of association, NAT2 [53,89,90], as well as the difficulties inherent to haplotype assigning when several polymorphisms are analyzed in the same gene, as is the case of NAT2 [91].
factors should be taken into consideration in the genetic assessment of NSAID-induced hepatotoxicity.

The combination of GWAS and CGAS studies indicate clear associations of HLA genes with drug-induced hepatotoxicity, thus suggesting an immune response [43]. However, many mechanisms involved in the immune response to drugs remain to be explored in mechanistically-based CGAS. Phenomic and genomic factors involved in hypersensitivity reactions to NSAIDs have recently been reviewed [92,93] and these should be assessed in NSAID-induced hepatotoxicity because, as stated in the mechanistic models, immune reactions of NSAID metabolites may trigger the hepatotoxicity events [41,42]. Drug-induced hepatitis shares many of the features of cell-mediated reactions, such as eosinophilia, atypical peripheral blood lymphocytes and a liver T-cell infiltrate. Drugs involved include methimazole, diclofenac, ibuprofen and occasionally any NSAID [92]. Alterations in genes coding for enzymes involved in histamine homeostasis, which are related to the development or the clinical presentation of immune diseases, such as those of the enzymes diamine oxidase or histidine decarboxylase [94-98], remain to be analyzed.

Two particular areas of the research of interest at present are transcriptomics and metabolomics. It has recently been shown that acetaminophen causes a transcriptome signature characterized by downregulation of oxidative phosphorylation genes [99]. The availability of rapid and affordable analyses of blood transcriptome in patients with drug-induced hepatotoxicity opens up new and exciting possibilities in their assessment. In addition to genomic, transcriptomics and phenomic analyses, because NSAIDs are extensively metabolized in humans, the metabonomic [100] approach to biological analysis in NSAID-induced hepatotoxicity should contribute significantly to improving our knowledge of the mechanisms involved in the first stages of liver injury.

Acknowledgements

The authors thank J McCue for assistance in language editing.

Declaration of interest

The work of the authors’ laboratory is funded by a number of grants. JAG Agúndez is supported by Grants PS09/00943 and RETICS RD07/0064/0016; E García-Martín is supported by PS09/00469 while RT Andrade is supported by PS 09/01384 and CIBERehd from the Instituto de Salud Carlos III, Madrid, Spain. JAG Agúndez is also supported by GR10068 from Junta de Extremadura, Spain while RT Andrade is supported by grant CTS-6470 from Consejería de Salud, Junta de Andalucía, Spain. The authors are also financed in part with FEDER funds from the European Union.

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Assessment of NSAID-induced hepatotoxicity


* A comprehensive review of metabolomics.

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