Minireview

Roles of co-factors in drug-induced liver injury: drug metabolism and beyond

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Abbreviation list

ADS, antioxidant defense system; APAP, acetaminophen; BSEP, bile salt export pump; BSO, buthionine sulfoximine; CYP, Cytochrome P450; DILI, drug-induced liver injury; EGFR, epidermal growth factor receptor; GSH, glutathione; GST, glutathione S-transferase; HLA, human leukocyte antigens; IL, interleukin; INH, isoniazid; LPS, lipopolysaccharide; MDSC, myeloid-derived suppressor cell; MRP, multidrug resistance-associated protein; NAFLD, nonalcoholic fatty liver disease; NLRP3, NLR family pyrin domain containing 3; Nrf2, nuclear erythroid factor type 2; PXR, pregnane X receptor; RIF, rifampicin; ROS, reactive oxygen species; TB, tuberculosis; TNF- α , tumor necrosis factor- α ; TVX, trovafloxacin; UGT, UDP-glucuronosyltransferase; VEGFR, vascular endothelial growth factor receptor

Abstract

Drug-induced liver injury (DILI) remains one of the major concerns for healthcare providers and patients. Unfortunately, it is difficult to predict and prevent DILI in the clinic because detailed mechanisms of DILI are largely unknown. Many risk factors have been identified for both “intrinsic” and “idiosyncratic” DILI, suggesting that co-factors are an important aspect in understanding DILI. This review outlines the co-factors that potentiate DILI and categorizes them into two types: (1) the specific co-factors that target metabolic enzymes, transporters, antioxidation defense, immune response and liver regeneration; and (2) the general co-factors that include inflammation, age, gender, comorbidity, gut microbiota and lifestyle. The underlying mechanisms by which co-factors potentiate DILI are also discussed.

Significance statement

This review summarizes the risk factors for DILI, which can be used to predict and prevent DILI in the clinic. This work also highlights the gaps in the DILI field and provides future perspectives on the roles of co-factors in DILI.

1. Introduction

Drug-induced liver injury (DILI) is an important health problem in the clinic. The incidence of DILI is approximately 14 to 19 cases per 100,000 population, and this number is underestimated because of the limited reporting system (Sgro et al., 2002; Björnsson et al., 2013; Ahmad and Odin, 2017). Most patients who suffer from DILI recover after discontinuation of the drug. However, some patients may develop severe DILI, leading to hospitalization, liver transplantation, and even death (Fontana et al., 2014). In addition, DILI is one of the most common causes of drug withdrawal from the market (Björnsson, 2015; Hoofnagle and Björnsson, 2019). Between 1976 and 2005, 28 drugs had been withdrawn from the market in the USA, among which 6 were removed because of hepatotoxicity (Kaitin, 2005; Babai et al., 2021).

Unfortunately, the detailed mechanisms of DILI are unclear for most drugs, which make it challenging for the prevention of DILI in the clinic. Clinical and preclinical studies have identified many co-factors of DILI, which are patient-specific attributes that increase the patient's susceptibility to DILI and/or contribute to the deterioration of DILI. Co-factors can affect both intrinsic and idiosyncratic types of DILI. The intrinsic DILI is dose-dependent and predictable (Kullak-Ublick et al., 2017; Hoofnagle and Björnsson, 2019). In addition, intrinsic DILI usually has a short latency period, and is reproducible in animal models (Sandhu and Navarro, 2020). The most well-known example of intrinsic DILI is caused by acetaminophen (APAP), which accounts for >50% cases of acute liver failure in the USA (Reuben et al., 2016). Even though APAP-induced liver injury belongs to the intrinsic type of DILI which is dose-dependent and predictable, there are also co-factors that make patients sensitive to APAP

hepatotoxicity such as diabetes and alcohol consumption (Zimmerman and Maddrey, 1995; Aubert et al., 2012). Co-factors have also been identified for the idiosyncratic type of DILI, which is dose-independent and unpredictable (Hoofnagle and Björnsson, 2019; Sandhu and Navarro, 2020). For example, isoniazid (INH)-induced idiosyncratic DILI can be potentiated by rifampicin (RIF) (Steele et al., 1991; Chowdhury et al., 2006; Li et al., 2013). In addition, deficiency of *N*-acetyltransferase 2 also increases the risk of INH hepatotoxicity (Huang et al., 2002; Nicoletti et al., 2021). These data suggest that co-factors play an important role in the development of DILI.

In the current review, we discuss the co-factors that potentiate DILI, which include specific co-factors that target drug metabolizing enzymes, transporters, endobiotic metabolism, antioxidation defense, immune response, and tissue repair; and general co-factors such as inflammation, age, gender, comorbidity, gut microbiota, nutrition, and lifestyle. We also discuss the underlying mechanisms of co-factors that modulate DILI.

2. Co-factors that alter the exposure of drug and its metabolites to the liver and potentiate DILI

2.1. Variations of Phase I drug metabolizing enzymes and DILI

Most drugs are metabolized in the liver through two phases of metabolic process. In Phase I metabolism, parent drugs go through various reactions including oxidation, reduction, and hydrolysis. Oxidation is the most common reaction in Phase I metabolism, which is catalyzed by enzymes including cytochrome P450 (CYP), flavin-containing monooxygenase, and monoamine

oxidase. Among these Phase I enzymes, CYPs are the most common family and have been extensively studied for their roles in DILI.

Induction of CYP enzymes can increase the risk of DILI if the CYP-mediated metabolites are toxic. A series of clinical trials revealed hepatotoxicity in subjects pretreated with RIF followed by ritonavir-containing antiretroviral drugs (Nijland et al., 2008; Haas et al., 2009; Schmitt et al., 2009). RIF is a human specific activator of pregnane X receptor (PXR) that upregulates CYP3A4 (Bertilsson et al., 1998; Lehmann et al., 1998; Goodwin et al., 1999). Using the PXR-humanized mouse model, it was found that RIF increased the CYP3A4-dependent activation pathways of ritonavir to generate toxic metabolites, leading to oxidative stress and hepatocellular injury (Shehu et al., 2019). Cobicistat is an analogue of ritonavir that is also used for antiretroviral therapy. It is generally believed that the safety profile of cobicistat is better than that of ritonavir (Xu et al., 2010; Renjifo et al., 2015). However, activation of the two xenobiotic receptors that induce CYP3A4, PXR and constitutive androstane receptor, potentiates cobicistat hepatotoxicity through the CYP3A4-dependent pathways, suggesting that CYP3A4 inducers are risk factors for cobicistat hepatotoxicity (Shehu et al., 2021). Indeed, CYP inducers as co-factors that potentiate DILI have already been determined for many other drugs, such as valproic acid and APAP (Gopaul et al., 2003; Cheng et al., 2009a).

Other than CYP induction, CYP inhibition can also potentiate DILI if the parent drug is hepatotoxic. Many clinically used drugs as well as chemicals from environment and herbal supplements inhibit CYPs, which decrease the metabolism of the victim drug and increase its

exposure to the liver and potentially lead to hepatotoxicity (Gorski et al., 2004; Lynch and Price, 2007; Causevic-Ramosevac and Semiz, 2013; Wanwimolruk and Prachayasittikul, 2014). For example, atorvastatin hepatotoxicity is boosted by grapefruit juice because it inhibits the CYP3A4-mediated detoxification of atorvastatin (Lilja et al., 1999; Causevic-Ramosevac and Semiz, 2013). Additionally, a dramatic decrease of CYP activity was observed in mice under liver injury condition (Bao et al., 2020; Bao et al., 2021), suggesting that dose adjustment is needed when other drugs are applied, especially for a parent drug that is hepatotoxic.

Furthermore, genetic polymorphisms of CYPs can alter CYP function and contribute to DILI. In ticlopidine-induced liver injury, **1H* and **1J* haplotypes of CYP2B6 increase the production of toxic metabolites of ticlopidine and boost the risk of ticlopidine hepatotoxicity (Ariyoshi et al., 2010). In another case, a loss-of-function mutation of *CYP2B6*6* was found in patients with efavirenz-induced liver injury, which decreased CYP2B6-dependent metabolism of efavirenz and resulted in efavirenz accumulation in the liver (Yimer et al., 2012). Because CYPs are important in drug metabolism and have high individual variations, genetic polymorphisms of CYPs as well as their induction and/or inhibition should be considered before prescribing patients a potential hepatotoxic drug that is metabolized by CYPs.

2.2. Variations of Phase II drug metabolizing enzymes and DILI

Phase II drug metabolizing enzymes include UDP-glucuronosyltransferase (UGT), sulfotransferase, glutathione S-transferase (GST), etc. These enzymes catalyze the conjugation of substrates to hydrophilic groups like UDP glucuronic acid, sulfate, and glutathione (GSH).

Substrates for Phase II enzymes are usually generated from Phase I metabolism or sometimes parent drugs. The products of Phase II metabolism are generally less active and more readily eliminated.

For most drugs, Phase II metabolism is a detoxification process and inhibition of Phase II metabolism leads to adverse outcomes. Pexidartinib is a tyrosine kinase inhibitor approved for treating adults with symptomatic tenosynovial giant cell tumor that is not responsive to surgical treatment (Tap et al., 2019). However, pexidartinib has a boxed warning of hepatotoxicity. Pexidartinib is metabolized by CYP3A4 and UGT1A4 to oxidative and glucuronide metabolites accordingly for elimination. It has been found that probenecid, a nonspecific UGT inhibitor, slows down pexidartinib elimination, leading to its accumulation and liver injury (Lamb, 2019). In addition, phenobarbital and phenytoin potentiate APAP hepatotoxicity partially due to their inhibition on APAP glucuronidation, one of the major detoxification and elimination pathways of APAP (Kostrubsky et al., 2005).

Phase II metabolism may also mediate drug bioactivation and potentiate DILI (Hinson et al., 1995). For example, diclofenac is a widely prescribed analgesic associated with idiosyncratic liver injury. The exact mechanism of diclofenac-induced liver injury is not well understood, but the metabolism of diclofenac by UGT2B7 to form diclofenac acyl glucuronide might contribute to such toxicity (Laine et al., 2009). Indeed, *UGT2B7*2* polymorphic variant is associated with diclofenac hepatotoxicity (Daly et al., 2007). *UGT2B7*2* has an increased glucuronidation

activity which leads to a surge in the formation of diclofenac acyl glucuronide and liver injury (Duguay et al., 2004; Daly et al., 2007).

2.3. Variations of drug transporters and DILI

Hepatic transporters including the solute carrier family and the ATP-binding cassette family are critical for distribution and elimination of drugs and endobiotics like bile acids and bilirubin. Influx transporters, such as organic anion transporting polypeptide 1B1/1B3 and Na⁺-taurocholate co-transporting polypeptide, are members of solute carrier family. Efflux transporters are mainly from the ATP-binding cassette family including multidrug resistance-associated proteins (MRP), bile salt export pump (BSEP), breast cancer resistance protein (BCRP), and P-glycoprotein (Roth and Lee, 2017). Similar to drug metabolizing enzymes, the functions of transporters can be altered by inducers, inhibitors, and genetic polymorphisms.

Inhibition of efflux transporters is a common risk factor for DILI. Emodin, an active anthraquinone in herbal medicines, is hepatotoxic and its toxicity can be exacerbated by probenecid, which inhibits the MRP2-mediated excretion of emodin from hepatocytes (Wu et al., 2018). Troglitazone, an antidiabetic and anti-inflammatory drug, was withdrawn from the market because of hepatotoxicity (Funk et al., 2001; Jackson et al., 2018). Troglitazone causes cholestatic liver injury because (1) this drug inhibits BSEP, the major efflux transporter of bile acids (Funk et al., 2001) and (2) troglitazone suppresses the function of farnesoid X receptor, a transcription factor that upregulates BSEP expression (Jackson et al., 2018).

Loss-of-function mutations of efflux transporters are also risk factors for DILI. MRP2 is associated with diclofenac-induced liver injury (Daly et al., 2007). By comparing the genotypes of the patients who encountered diclofenac-induced liver injury to those without DILI, the *C-24T* variant of MRP2 was identified, which reduces MRP2 expression, leading to the accumulation of diclofenac and its metabolites in hepatocytes (Haenisch et al., 2007). MRP2 is also responsible for bilirubin efflux and genetic deficiency of MRP2 causes Dubin-Johnson syndrome, characterized by hyperbilirubinemia (Keppler, 2014). In addition, the *I331T>C* mutation of BSEP increases the susceptibility to contraceptive-induced cholestasis because estrogen and progesterone in contraceptive pill inhibit BSEP-mediated bile acid excretion and the loss-of-function mutation of BSEP exacerbates bile acid accumulation (Meier et al., 2008).

3. Co-factors that interact with the molecular targets of drug and its metabolites in the liver and potentiate DILI

Drugs are designed to specifically target molecules of interest to generate therapeutic effects. However, drugs and/or their metabolites may interact with other molecules leading to off-target effects and even toxicity (Rudmann, 2012). Compared to the therapeutic targets of drugs, which are known for most drugs, the toxic targets of drugs and their metabolites are understudied and largely unknown. For example, the molecular targets in hepatocytes are not clear for most hepatotoxic drugs after they enter hepatocytes (**Figure 1**).

The applications of omics facilitate the screening of off-targets of drugs (Wang et al., 2017; Paananen and Fortino, 2020). RIF and INH are the first line drugs for the treatment of

tuberculosis (TB) by inhibiting TB RNA polymerase and cell wall synthesis, respectively (Schulz and Zillig, 1981; Quemard et al., 1991). However, co-treatment with RIF and INH causes liver damage (Steele et al., 1991; Chowdhury et al., 2006; Li et al., 2013). Recent metabolomic studies revealed that co-treatment with RIF and INH causes the accumulation of protoporphyrin IX, a hepatotoxin, by inducing delta-aminolevulinate synthase 1 and suppressing ferrochelatase in the liver (Li et al., 2013; Sachar et al., 2016), suggesting that the heme biosynthesis pathway is the off-target of RIF and INH in hepatocytes. INH also interacts with CD38 in Kupffer cells and hepatic stellate cells, leading to the formation of INH-NAD and AcINH-NAD adducts, which potentially contribute to liver injury because of the critical roles of NAD^+ in cellular functions (Zhu et al., 2020; Amjad et al., 2021).

Identification of drug off-targets in the liver can be used to prevent DILI by avoiding the co-factors that deliver the second hit on the pathways that are related to the off-targets of the victim drug (**Figure 1**). Reduced GSH plays an important role in antioxidant defense as well as in regulation of cellular metabolic functions including gene expression, signal transduction, cell proliferation and apoptosis (Aquilano et al., 2014). GSH is the primary target of *N*-acetyl-*p*-benzoquinone imine, the reactive metabolite of APAP in the liver, and overdose of APAP leads to GSH depletion and cellular injury (Siegers, 1978; Chiba and Pang, 1995; Li et al., 2011). Therefore, any co-factors that cause GSH deficiency will increase the risk of APAP toxicity, such as buthionine sulfoximine (BSO), a potent inhibitor of GSH synthesis (Tamai et al., 2017). On the other hand, treatment with *N*-acetylcysteine, a precursor of GSH, rescues APAP hepatotoxicity (Ruffmann and Wendel, 1991). Unfortunately, the off-targets of most DILI drugs in the liver remain unknown (Wang et al., 2017; Paananen and Fortino, 2020). Therefore, there is

an urgent need to explore this field in order to develop mechanism-based approaches to predict and prevent DILI.

4. Co-factors that disrupt cell defense, adaptation, and tissue repair in the liver and potentiate DILI

4.1. Antioxidant defense and DILI

The antioxidant defense system (ADS) combats oxidative stress by reacting with reactive oxygen species (ROS) and prevents ROS-mediated cellular damage (Jaeschke et al., 2012; Ray et al., 2012). ADS consists of peptides and enzymes. GSH, a small peptide, reacts directly with ROS and protects cells against toxicity from excessive amounts of electrophiles, hydroxyl radicals, and superoxide (Birben et al., 2012; Jaeschke et al., 2012; Ray et al., 2012). ADS enzymes include superoxide dismutase and catalase (Birben et al., 2012). In addition, GSTs, a family of Phase II enzymes, also contribute to ADS by catalyzing the conjugation of GSH to a wide variety of electrophilic compounds and protecting cellular macromolecules (Jakoby, 1985; Townsend and Tew, 2003).

For drugs that damage hepatocytes via oxidative stress, co-factors that promote the formation of ROS or impair ADS will potentiate DILI. BSO inhibits glutamate cysteine ligase (GCL), a critical enzyme in GSH synthesis, and thus suppresses GSH production. Methimazole is a widely used antithyroid drug, but it can cause hepatotoxicity by unknown mechanisms. Studies found that pretreatment with BSO depletes GSH in the liver and potentiates methimazole hepatotoxicity in mice (Mizutani et al., 1999; Mizutani et al., 2000). BSO also amplifies

APAP-induced liver injury (Tamai et al., 2017). In addition, genetic polymorphisms of ADS encoding genes potentiate DILI. C to T substitution mutation at position 47 of superoxide dismutase and GST-M1 null genotype increase the risk of anti-TB drug-induced liver injury (Huang et al., 2007). The loss-of-function mutations of GST also increase the risk of tacrine and troglitazone-induced liver injury (Simon et al., 2000; Okada et al., 2011).

The nuclear erythroid factor type 2 (Nrf2) regulates ADS (Enomoto et al., 2001; Li and Kong, 2009; Telkoparan-Akillilar et al., 2019). Nrf2 activation induces the expression of ADS enzymes and promotes the detoxification and cytoprotective process. On the other hand, Nrf2 inhibitors undermine ADS and potentiate DILI. In mice co-treated with APAP and brusatol, a Nrf2 inhibitor, more serious liver injury together with a lower GSH level and a higher ROS level were observed when compared to that of APAP alone group (Chan et al., 2001; Li et al., 2019).

4.2. Immune response and DILI

Apart from direct damage, abnormal activation of immune system also potentiate DILI. Small molecular drugs are unlikely to trigger immune response (Mak and Uetrecht, 2017). However, some reactive metabolites of drugs can bind to endogenous proteins and the drug-protein adducts trigger immune response (Mak and Uetrecht, 2017). Immune tolerance counteracts the immune response activated by neoantigen resulting in no apparent or mild liver injury in most patients (Uetrecht, 2007). There are mainly two mechanisms for immune tolerance in the liver: (1) antigen presenting cells in the liver produce various inhibitory cytokines like interleukin (IL) 10 and transforming growth factor beta 1, resulting in poor T cell activation (Erhardt et al., 2007;

Crispe, 2014); and (2) a suppressive network can be generated by myeloid-derived suppressor cells (MDSCs), which activate regulatory T cells and inhibits effector T cells (Chen et al., 2011). However, a small percentage of patients still develop strong immune response and cause severe liver injury, which might be due to additional environmental or genetic factors that modulate immune response.

Factors that impair immune tolerance can exacerbate immune-mediated DILI. An immune tolerance impaired mouse model has been generated by depleting the MDSCs cells (Chakraborty et al., 2015). Treatment with halothane, a drug that induces allergic hepatitis, developed hepatotoxicity in MDSCs depleted mice, but not in wild-type mice (Chakraborty et al., 2015), suggesting the protective role of immune tolerance in DILI. In addition to MDSCs, immune checkpoints CTLA-4 and PD-1 also modulate immune tolerance. Immune checkpoint inhibitors used in cancer therapy can suppress immune tolerance and thus increase the risk of immune-mediated DILI (Sznol et al., 2017). In patients co-treated with nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4), a high rate of hepatic adverse events occurred (Sznol et al., 2017). Preclinical studies also provided evidence for the role of immune tolerance inhibitors in DILI. In PD-1-null mice co-treated with amodiaquine and anti-CTLA4 antibody, a more severe liver injury was noted when compared to amodiaquine monotherapy group (Metushi et al., 2015).

Polymorphisms of human leukocyte antigens (*HLA*) have been identified as risk factors for immune-mediated DILI (Castiella et al., 2014). *HLA-B*5701* was found to be significantly

associated with flucloxacillin-induced liver injury (Daly et al., 2009). A later study confirmed the role of *HLA-B*5701* and suggested that *HLA-B*5703* is also associated with flucloxacillin hepatotoxicity (Nicoletti et al., 2019). More associations between *HLA* polymorphisms and DILI have been revealed such as *HLA-DRB1*0701* for lapatinib hepatotoxicity and *HLA-DRB1*1501* for lumiracoxib hepatotoxicity (Singer et al., 2010; Spraggs et al., 2011). These specific associations may result from certain *HLA* haplotypes having a high genetic predisposition to both the adaptive immune response and presentation of hapten-protein adducts required in DILI (Castiella et al., 2014).

4.3. Tissue repair and DILI

The liver has a remarkable regenerating capacity. After partial hepatectomy in mice, typically removal of two-thirds of the liver, hepatic mass is back to the original size in ~ 7 days (Michalopoulos, 2010). The APAP overdose mouse model that causes hepatocellular necrosis is frequently used to study liver regeneration in DILI, in which compensatory hepatocellular regeneration begins in the necrotic zones and newly proliferated cells replace the dead cells (Jaeschke et al., 2012; Bhushan et al., 2014). It remains unclear how injured hepatocytes communicate with their neighborhood healthy cells, but the involvement of extracellular vesicles has been proposed (Cho et al., 2018; Umbaugh and Jaeschke, 2021). The regeneration is critical for the final outcome and if regeneration fails, liver failure occurs (Mehendale, 2005). However, there are factors that can inhibit liver regeneration and thus potentiate DILI.

Growth factors play a critical role in liver regeneration. Epidermal growth factor receptor (EGFR) is involved in liver regeneration and its ligands act as mitogens that trigger hepatocyte proliferation (Michalopoulos and Khan, 2005; Michalopoulos, 2007). Inhibition of EGFR by canertinib at 12 hours post APAP treatment impaired liver regeneration which led to progression of injury and increased fatality in mice (Bhushan et al., 2017). In addition to EGFR, inhibition of vascular endothelial growth factor receptor (VEGFR) also results in impairment of liver regeneration (Donahower et al., 2006a). The expression of VEGF-A protein and its receptor was elevated after the initiation of APAP-induced liver injury in mice, suggesting the VEGFR signaling pathway contributes to liver regeneration (Donahower et al., 2006b; Papastefanou et al., 2007; Kato et al., 2011). A follow-up study revealed that VEGFR inhibitor SU5416 diminished hepatocyte regeneration in APAP-induced liver injury mouse model, which provided further evidence for the role of VEGFR in liver regeneration (Donahower et al., 2006a). These data suggest that growth factor inhibitors should be cautiously used when combined with hepatotoxic drugs.

In addition to growth factors, the regeneration process in the liver is also modulated by cytokines and chemokines, such as IL-6 and macrophage inflammatory protein-2 (Hogaboam et al., 1999; James et al., 2003). Knockout of IL-6 or macrophage inflammatory protein-2 receptor CXCR2 abrogates the protective effects against liver damage and increases the mortality in APAP overdose mouse model (Hogaboam et al., 1999; James et al., 2003), indicating that co-factors that decrease IL-6 level or act as CXCR2 antagonists may exacerbate DILI. Furthermore, the sympathetic nervous system also regulates liver regeneration (Oben and Diehl, 2004). By analyzing the clinical cases of APAP-induced liver injury in the FDA database, it was found that

co-administration of APAP with sympathetic stimulants was associated with the increased likelihood of fatality (Suzuki et al., 2009), suggesting that sympathetic stimulants should be considered as co-factors for DILI.

5. General co-factors that potentiate DILI

5.1. Inflammation and DILI

Co-existing inflammation and the onset of an inflammatory episode increase DILI susceptibility (Shaw et al., 2010). Inflammation is usually triggered by bacteria or virus infection, as well as pathophysiological changes including necrotic cell death. The most common inflammatory agent is lipopolysaccharide (LPS), the endotoxin from gram-negative bacteria (Hamesch et al., 2015). Trovafloxacin (TVX), an antibacterial quinolone agent, causes hepatotoxicity by unknown reasons. Interestingly, an inflammatory episode increases the risk of TVX-induced liver injury (Shaw et al., 2010). In mice and rats pre-treated with a nontoxic dose of TVX followed by a non-toxic dose of LPS, severe hepatocellular lesions were observed (Waring et al., 2006; Shaw et al., 2007). Peptidoglycan-lipoteichoic acid, an inflammatory agent extracted from gram-positive bacteria, also potentiates TVX-induced liver injury (Shaw et al., 2009). These data indicate that inflammation is a risk factor for TVX hepatotoxicity.

In addition to TVX, animal studies have revealed that LPS-induced inflammation potentiated hepatotoxicity of many other drugs including ranitidine (Luyendyk et al., 2003), sulindac (Zou et al., 2009b), chlorpromazine (Buchweitz et al., 2002), halothane (Dugan et al., 2010), amiodarone (Lu et al., 2012) and diclofenac (Deng et al., 2006). LPS interacts with inflammasomes, the

multiprotein complexes responsible for the activation of inflammatory responses (Schroder et al., 2012). NLR family pyrin domain containing 3 (NLRP3) is a component of the inflammasome, and activation of NLRP3 triggers proinflammatory signals and hepatocyte pyroptosis (Wree et al., 2014). Treatment with LPS potentiated carbamazepine hepatotoxicity in WT mice, but not in Nlrp3-null mice, indicating the pivotal role of NLRP3 and inflammasomes in carbamazepine-induced liver injury (Wang et al., 2019).

In humans under physiological condition, the serum LPS level is relatively low because the epithelial membranes in the intestines retain LPS inside the gut and prevent them entering bloodstream (Salden and Bas, 1994; Wassenaar et al., 2018). Unfortunately, many factors can destruct intestinal barrier, leading to an increase of LPS level in bloodstream and increase the risk of DILI (Ghosh et al., 2020). Alcohol consumption, obesity, and high fat diet have been considered as risk factors of DILI because they increase intestinal permeability and blood levels of LPS (Bala et al., 2014; Boutagy et al., 2016). However, convincing data from the clinic are unavailable to ascertain whether LPS should be considered as a bona fide risk factor for DILI. Given the fact that many factors may increase LPS levels in the blood, more clinical studies are needed to determine the impact of LPS on DILI.

In addition to LPS produced from bacteria, virus particles also induce inflammation and exacerbate DILI. Cheng and colleagues employed viral RNA mimetic polyI:C to mimic viral infection-induced inflammation and found that polyI:C significantly potentiated halothane-induced hepatocellular injury (Cheng et al., 2009b). In addition, patients with human

immunodeficiency virus (HIV) have a high incidence of DILI when receiving antiretroviral therapy, especially in HIV patients co-infected with hepatitis B or C (Aceti et al., 2002; Pignataro et al., 2004; Hunt, 2012). One possible explanation is that inflammation triggered by hepatitis B or C sensitizes HIV patients to DILI (Ganey et al., 2004).

The exact mechanism by which inflammatory stress potentiates DILI is not fully understood. Nevertheless, it has been speculated that tumor necrosis factor- α (TNF- α) plays a critical role in the development of DILI (Shaw et al., 2007). TNF- α is a proinflammatory cytokine and the serum concentration of TNF- α is significantly increased after LPS exposure (Shaw et al., 2007; Zou et al., 2009a; Shaw et al., 2010). TNF- α potentiates apoptotic cell death of hepatocytes especially under the condition of GSH depletion (Colell et al., 1998; Matsumaru et al., 2003; Yan et al., 2016). In addition, TNF- α has a positive correlation with other cytokines, such as IL-18 and interferon γ , which may team up amplifying the impact of inflammatory stress on liver damage (Shaw et al., 2009). Furthermore, TNF- α enhances the activation of neutrophils and contributes to the development of DILI (Shaw et al., 2009). Moreover, TNF- α increases the level of plasminogen activator inhibitor-1, which inhibits fibrinolysis, leading to fibrin accumulation, hypoxia, and liver damage (Watkins and Seeff, 2006; Shaw et al., 2009; Shaw et al., 2010).

5.2. Age and DILI

Age, either young or old, can be a risk factor for DILI for certain medications. Compared to patients between 25 to 34 years old, the rate of INH-induced liver injury increased two-fold in patients between 35 to 49 years old, and five-fold in patients above 50 years old (Fountain et al.,

2005; Andrade et al., 2019). The underlying mechanisms for the age-related INH-induced liver injury include a decline in liver regeneration capacity, concurrent diseases, and age-related changes of INH pharmacokinetics in the elderly (Schmucker et al., 2011; Lin et al., 2016; Mach et al., 2016). Opposite to INH-induced liver injury, the young age is a risk factor for valproic acid hepatotoxicity, especially for children < 2 years old, which is partially due to the differences of valproic acid metabolism and disposition between adults and children (Bryant and Dreifuss, 1996; Andrade et al., 2019).

5.3. Gender and DILI

It is commonly believed that women are at a higher risk of developing more severe DILI than men (Russo et al., 2004; Lucena et al., 2009). Indeed, the risk of immune-mediated DILI is significantly higher in women than men. Distinct hormone composition between males and females may contribute to different susceptibility to DILI. Culture of spleen cells from female mice showed fewer regulatory T cells and higher pro-inflammatory cytokines than males, which is partially due to the gender difference of 17 β -estradiol (Cho et al., 2013). In addition, pre-treatment with progesterone potentiates halothane-induced liver injury because progesterone increases the level of hepatic proinflammatory cytokines and chemokines (Toyoda et al., 2011). Moreover, gender differences in drug metabolizing enzymes and transporters may also contribute to DILI susceptibility, as CYP2E1 and P-glycoprotein have higher activities in men than in women; while women have a higher CYP3A4 activity than men (Meibohm et al., 2002; Schwartz, 2003).

5.4. Comorbidity and DILI

Hepatitis B and C are well-known risk factors for DILI, which create a pro-inflammatory environment and potentiate DILI (Schenker et al., 1999; Burdette et al., 2012; Kim et al., 2016). HCV patients with anti-TB treatment had a 31.7% incidence of DILI, and the incidence was even higher at 75% in those co-infected with HBV and HCV, while the DILI incidence for control group (infected with TB only) was 10% (Kim et al., 2016). In addition to hepatitis B and C, obesity, diabetes, and nonalcoholic fatty liver disease (NAFLD) are also risk factors for DILI. Both obesity and diabetes increase hepatic oxidative stress, which can deteriorate mitochondrial function and increase susceptibility to hepatotoxic drugs (Koliaki and Roden, 2013; Pais et al., 2014). Patients with NAFLD have increased CYP2E1 activity and dysfunctional mitochondrial respiratory chain in hepatocytes, leading to an aggressive production of ROS and a high risk of DILI (Aubert et al., 2012; Fromenty, 2017). Furthermore, concurrent diseases with the disorder of bile acid homeostasis can potentiate DILI (Gomez-Ospina et al., 2016). It has been found that deficiency of farnesoid X receptor causes deoxycholic acid accumulation, which increases the risk of APAP-induced liver injury (Yan et al., 2021).

5.5. Gut microbiota and DILI

The bacteria in the gut have their own metabolic system and contribute to drug metabolism and disposition in the host (Sousa et al., 2008; Enright et al., 2016; Yip et al., 2018). Tacrine-induced liver injury is associated with the increases of gut bacteria including *Lactobacillus*, *Bacteroides* and *Enterobacteriaceae* (Yip et al., 2018). These three strains of bacteria have deglucuronidation capacity, which slow down tacrine elimination, increase tacrine plasma concentration, and

therefore potentiate tacrine hepatotoxicity (Yip et al., 2018). In addition, the metabolites from gut microbiota can enter systemic circulation and disturb liver functions. P-cresol is a bacterial metabolite produced by *Clostridium difficile*, which competes with APAP for the detoxification process mediated by sulfotransferase 1A1, leading to APAP accumulation and liver injury (Clayton et al., 2009). Another bacterial metabolite PPD, produced by *Escherichia coli* and *Citrobacter freundii*, depletes GSH in hepatocytes and exacerbates APAP-induced liver injury (Gong et al., 2018; Niu and Chen, 2020). Overall, gut bacteria as well as their metabolites can alter drug metabolism and host responses and increase the risk of DILI.

5.6. Lifestyle and DILI

Both malnutrition and overnutrition are risk factors for DILI. Malnutrition is significantly associated with anti-TB drug-induced liver injury (Mehta and therapeutics, 1990; Makhoul et al., 2008; Ali et al., 2020). Malnutrition-mediated alteration of drug metabolism, antioxidant defense, and tissue repair may contribute to the hepatotoxicity of anti-TB drugs, but more mechanistic studies are needed (Speerhas, 1995; Makhoul et al., 2008; Villanueva-Paz et al., 2021). In addition, individuals who have a high fat diet are likely to develop liver inflammation because the fat-abundant diet contributes to the overgrowth of intestinal bacteria, which can damage intestinal integrity and increase the exposure of inflammatory agents to the liver (Utzeri and Usai, 2017). Moreover, a high fat diet and overnutrition increase the possibility to develop obesity and NAFLD, leading to an increase in hepatocellular oxidative stress and the risk of DILI (Koliaki and Roden, 2013).

Alcoholic drinks including beers, wines, and liquors are very common in daily life, which can increase the risk of DILI by CYP2E1 induction (Zimmerman and Maddrey, 1995), GSH suppression (Lauterburg and Velez, 1988), disruption of gut microbiota (Mutlu et al., 2009), and promotion of liver inflammation (Fujimoto et al., 2000; Thakur et al., 2007). Alcohol-APAP syndrome has been reported since 1980s, in which alcohol increases APAP hepatotoxicity by inducing the expression of CYP2E1 as well as suppressing hepatic GSH synthesis (Zimmerman and Maddrey, 1995). The induction of CYP2E1 by alcohol increases the production of reactive metabolite of APAP, while GSH depletion results in the failure of antioxidant defense (Zimmerman and Maddrey, 1995). In addition, chronic alcohol consumption has been identified as an independent predictor for mortality of APAP-induced liver injury (Schmidt et al., 2002). Chronic alcohol abuse also increases the risk of anti-TB drug-induced liver injury as 42.2% of DILI cases showed significant alcohol consumption (Gaude et al., 2015).

Cigarette may also potentiate DILI. A retrospective study found that tobacco is an independent risk factor for APAP-induced liver injury (Schmidt and Dalhoff, 2003). Benzothiazole in cigarette induces CYP1A2 (Xiao Dong et al., 1998), and pre-treatment with benzothiazole increased APAP bioactivation and hepatotoxicity (Seo et al., 2000). However, compared to alcohol consumption, the relationship between smoking and hepatotoxicity is less prevalent (Stine and Chalasani, 2017). Indeed, a decreased risk for anti-TB drug-induced liver injury was observed in active smokers when compared to non-smokers (Zaverucha-do-Valle et al., 2014), and a low association was identified between smoking and hepatotoxicity of flutamide, an antiandrogenic drug (Wada et al., 1999).

Illicit substances including cocaine and amphetamine-type stimulants also boost DILI. Clinical studies have observed mild to severe liver injury in cocaine users, which in part is due to norcocaine, a metabolite of cocaine that causes hepatic oxidative stress (Silva et al., 1991). In addition, 3,4-methylenedioxymethamphetamine, an amphetamine-type stimulant, can cause serious liver damage and even acute liver failure (Greene et al., 2003). Since drug abusers usually consume more than one addictive substance, the concurrent use of illicit drugs make the liver condition even worse (Pateria et al., 2013). Therefore, when a hepatotoxic medicine is prescribed to drug abusers, the vulnerable liver may not be able to tolerate the prescription and result in liver damage.

6. Summary and perspectives

The detail mechanisms of DILI are unknown for most drugs, but it is generally accepted that three steps are needed in the development of DILI: (1) exposure of drug and its metabolites to the liver; (2) interactions of drug and its metabolites with molecular targets in the liver, leading to cellular dysfunction and injury; and (3) cellular dysfunction and injury trigger cell defense, adaptation, and tissue repair, and inappropriate cell responses result in liver injury (**Figure 2**). Many co-factors that potentiate DILI have been identified, and the current review categorized them into two types, the specific and the general co-factors. Specific co-factors increase the risk of DILI by directly targeting a specific molecule that is involved in the three steps of liver injury, such as an inhibitor of drug metabolizing enzyme or transporter (**Figure 2**). General co-factors,

including inflammation, age, gender, comorbidity, gut microbiota, nutrition, and lifestyle, can potentiate DILI by affecting multiple molecules in the process of liver injury (**Figure 2**).

The risk factors for DILI can potentially be used to predict and prevent DILI, therefore improving the safety profile of medications. However, more studies are needed to define the mechanisms by which co-factors potentiate DILI. In addition, more co-factors for DILI are expected to be discovered based upon the identification of target molecules of drugs in the liver. Unfortunately, the molecular targets of most DILI drugs in the liver remain elusive. The applications of genomics, transcriptomics, proteomics, metabolomics, together with genetically engineered cellular and animal models facilitate the identification of target molecules of drugs in the liver (Chang et al., 2010; Strähle and Grabher, 2010; Gonzalez et al., 2015; Wang et al., 2017; Huang et al., 2019; Paananen and Fortino, 2020), which will provide mechanistic understanding of DILI and expedite the discovery of novel risk factors for DILI. Additionally, the artificial intelligence approaches also show a great potential for predicting DILI (Minerali et al., 2020; Vall et al., 2021).

In summary, co-factors for DILI are the attributes that increase DILI susceptibility and/or potentiate DILI progression. Co-factors affect DILI from various aspects including drug metabolism and disposition, drug-target interactions, and cellular responses. A better understanding of co-factors for DILI provides mechanistic understanding of DILI and allows for improved prediction and prevention of DILI.

Authorship Contributions

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References

- Aceti A, Pasquazzi C, Zechini B, De Bac C, and The LG (2002) Hepatotoxicity Development During Antiretroviral Therapy Containing Protease Inhibitors in Patients With HIV: The Role of Hepatitis B and C Virus Infection. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **29**.
- Ahmad J and Odin JA (2017) Epidemiology and Genetic Risk Factors of Drug Hepatotoxicity. *Clin Liver Dis* **21**:55-72.
- Ali N, Gupta N, and Saravu K (2020) Malnutrition as an important risk factor for drug-induced liver injury in patients on anti-tubercular therapy: an experience from a tertiary care center in South India. *Drug Discoveries & Therapeutics* **14**:135-138.
- Amjad S, Nisar S, Bhat AA, Shah AR, Frenneaux MP, Fakhro K, Haris M, Reddy R, Patay Z, Baur J, and Bagga P (2021) Role of NAD(+) in regulating cellular and metabolic signaling pathways. *Mol Metab* **49**:101195.
- Andrade RJ, Aithal GP, Björnsson ES, Kaplowitz N, Kullak-Ublick GA, Larrey D, and Karlsen TH (2019) EASL Clinical Practice Guidelines: Drug-induced liver injury. *Journal of Hepatology* **70**:1222-1261.
- Aquilano K, Baldelli S, and Ciriolo MR (2014) Glutathione: new roles in redox signaling for an old antioxidant. *Front Pharmacol* **5**:196.
- Ariyoshi N, Iga Y, Hirata K, Sato Y, Miura G, Ishii I, Nagamori S, and Kitada M (2010) Enhanced Susceptibility of HLA-mediated Ticlopidine-induced Idiosyncratic Hepatotoxicity by *CYP2B6* Polymorphism in Japanese. *Drug Metabolism and Pharmacokinetics* **25**:298-306.

- Aubert J, Begriche K, Delannoy M, Morel I, Pajaud J, Ribault C, Lepage S, McGill MR, Lucas-Clerc C, Turlin B, Robin M-A, Jaeschke H, and Fromenty B (2012) Differences in Early Acetaminophen Hepatotoxicity between Obese ob/ob and db/db Mice. *Journal of Pharmacology and Experimental Therapeutics* **342**:676.
- Babai S, Auclert L, and Le-Louët H (2021) Safety data and withdrawal of hepatotoxic drugs. *Therapies* **76**:715-723.
- Bala S, Marcos M, Gattu A, Catalano D, and Szabo G (2014) Acute Binge Drinking Increases Serum Endotoxin and Bacterial DNA Levels in Healthy Individuals. *PLoS One* **9**:e96864.
- Bao Y, Phan M, Zhu J, Ma X, Manautou JE, and Zhong X-b (2021) Alterations of cytochrome P450-mediated drug metabolism during liver repair and regeneration after acetaminophen-induced liver injury in mice. *Drug Metab Dispos*:DMD-AR-2021-000459.
- Bao Y, Wang P, Shao X, Zhu J, Xiao J, Shi J, Zhang L, Zhu H-J, Ma X, Manautou JE, and Zhong X-b (2020) Acetaminophen-Induced Liver Injury Alters Expression and Activities of Cytochrome P450 Enzymes in an Age-Dependent Manner in Mouse Liver. *Drug Metab Dispos* **48**:326.
- Bertilsson G, Heidrich J, Svensson K, Åsman M, Jendeberg L, Sydow-Bäckman M, Ohlsson R, Postlind H, Blomquist P, and Berkenstam AJPotNAoS (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. **95**:12208-12213.
- Bhushan B, Chavan H, Borude P, Xie Y, Du K, McGill MR, Lebofsky M, Jaeschke H, Krishnamurthy P, and Apte U (2017) Dual Role of Epidermal Growth Factor Receptor in

- Liver Injury and Regeneration after Acetaminophen Overdose in Mice. *Toxicological Sciences* **155**:363-378.
- Bhushan B, Walesky C, Manley M, Gallagher T, Borude P, Edwards G, Monga SPS, and Apte U (2014) Pro-Regenerative Signaling after Acetaminophen-Induced Acute Liver Injury in Mice Identified Using a Novel Incremental Dose Model. *Am J Pathol* **184**:3013-3025.
- Birben E, Sahiner UM, Sackesen C, Erzurum S, and Kalayci O (2012) Oxidative stress and antioxidant defense. *World Allergy Organ J* **5**:9-19.
- Björnsson ES (2015) Drug-induced liver injury: an overview over the most critical compounds. *Archives of Toxicology* **89**:327-334.
- Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, and Olafsson S (2013) Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. *Gastroenterology* **144**:1419-1425.
- Boutagy NE, McMillan RP, Frisard MI, and Hulver MW (2016) Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie* **124**:11-20.
- Bryant AE and Dreifuss FE (1996) Valproic acid hepatic fatalities. III. U.S. experience since 1986. **46**:465-469.
- Buchweitz JP, Ganey PE, Bursian SJ, and Roth RA (2002) Underlying Endotoxemia Augments Toxic Responses to Chlorpromazine: Is There a Relationship to Drug Idiosyncrasy? *Journal of Pharmacology and Experimental Therapeutics* **300**:460.
- Burdette D, Haskett A, Presser L, McRae S, Iqbal J, and Waris GJTJogv (2012) Hepatitis C virus activates interleukin-1 β via caspase-1-inflammasome complex. **93**:235.

- Castiella A, Zapata E, Lucena MI, and Andrade RJ (2014) Drug-induced autoimmune liver disease: A diagnostic dilemma of an increasingly reported disease. *World J Hepatol* **6**:160-168.
- Causevic-Ramosevac A and Semiz S (2013) Drug interactions with statins. *Acta Pharmaceutica* **63**:277-293.
- Chakraborty M, Fullerton AM, Semple K, Chea LS, Proctor WR, Bourdi M, Kleiner DE, Zeng X, Ryan PM, Dagur PK, Berkson JD, Reilly TP, and Pohl LR (2015) Drug-induced allergic hepatitis develops in mice when myeloid-derived suppressor cells are depleted prior to halothane treatment. *Hepatology* **62**:546-557.
- Chan K, Han X-D, and Kan YWJPotNAoS (2001) An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. **98**:4611-4616.
- Chang RL, Xie L, Xie L, Bourne PE, and Palsson BØ (2010) Drug Off-Target Effects Predicted Using Structural Analysis in the Context of a Metabolic Network Model. *PLOS Computational Biology* **6**:e1000938.
- Chen S, Akbar SMF, Abe M, Hiasa Y, and Onji M (2011) Immunosuppressive functions of hepatic myeloid-derived suppressor cells of normal mice and in a murine model of chronic hepatitis B virus. *Clinical & Experimental Immunology* **166**:134-142.
- Cheng J, Ma X, Krausz KW, Idle JR, and Gonzalez FJ (2009a) Rifampicin-Activated Human Pregnane X Receptor and CYP3A4 Induction Enhance Acetaminophen-Induced Toxicity. *Drug Metabolism and Disposition* **37**:1611.
- Cheng L, You Q, Yin H, Holt M, Franklin C, and Ju C (2009b) Effect of polyI:C cotreatment on halothane-induced liver injury in mice. *Hepatology* **49**:215-226.

- Chiba M and Pang KS (1995) Glutathione depletion kinetics with acetaminophen. A simulation study. *Drug Metab Dispos* **23**:622-630.
- Cho J, Kim L, Li Z, Rose NR, Talor MV, and Njoku DB (2013) Sex Bias in Experimental Immune-Mediated, Drug-Induced Liver Injury in BALB/c Mice: Suggested Roles for Tregs, Estrogen, and IL-6. *PLoS One* **8**:e61186.
- Cho YE, Song BJ, Akbar M, and Baek MC (2018) Extracellular vesicles as potential biomarkers for alcohol- and drug-induced liver injury and their therapeutic applications. *Pharmacol Ther* **187**:180-194.
- Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha DR, and Dhali GKJ (2006) Mitochondrial oxidative stress and permeability transition in isoniazid and rifampicin induced liver injury in mice. *45*:117-126.
- Clayton TA, Baker D, Lindon JC, Everett JR, and Nicholson JK (2009) Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *106*:14728-14733.
- Colell A, García-Ruiz C, Miranda M, Ardite E, Mari M, Morales A, Corrales F, Kaplowitz N, and Fernández-Checa JC (1998) Selective glutathione depletion of mitochondria by ethanol sensitizes hepatocytes to tumor necrosis factor. *Gastroenterology* **115**:1541-1551.
- Crispe IN (2014) Immune tolerance in liver disease. *Hepatology* **60**:2109-2117.
- Daly AK, Aithal GP, Leathart JBS, Swainsbury RA, Dang TS, and Day CP (2007) Genetic Susceptibility to Diclofenac-Induced Hepatotoxicity: Contribution of UGT2B7, CYP2C8, and ABCC2 Genotypes. *Gastroenterology* **132**:272-281.
- Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, Graham J, Park BK, Dillon JF, Bernal W, Cordell HJ, Pirmohamed M,

- Aithal GP, Day CP, for the Ds, and International SAEC (2009) HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nature Genetics* **41**:816-819.
- Deng X, Stachlewitz RF, Liguori MJ, Blomme EAG, Waring JF, Luyendyk JP, Maddox JF, Ganey PE, and Roth RA (2006) Modest Inflammation Enhances Diclofenac Hepatotoxicity in Rats: Role of Neutrophils and Bacterial Translocation. *Journal of Pharmacology and Experimental Therapeutics* **319**:1191.
- Donahower B, McCullough SS, Kurten R, Lamps LW, Simpson P, Hinson JA, and James LP (2006a) Vascular endothelial growth factor and hepatocyte regeneration in acetaminophen toxicity. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **291**:G102-G109.
- Donahower B, McCullough SS, Kurten R, Lamps LW, Simpson P, Hinson JA, James LPJAJoP-G, and Physiology L (2006b) Vascular endothelial growth factor and hepatocyte regeneration in acetaminophen toxicity. **291**:G102-G109.
- Dugan CM, MacDonald AE, Roth RA, and Ganey PE (2010) A Mouse Model of Severe Halothane Hepatitis Based on Human Risk Factors. *Journal of Pharmacology and Experimental Therapeutics* **333**:364.
- Duguay Y, Báár C, Skorpen F, and Guillemette C (2004) A novel functional polymorphism in the uridine diphosphate–glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. *Clinical Pharmacology & Therapeutics* **75**:223-233.
- Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, and Yamamoto MJTS (2001) High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity

- associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. **59**:169-177.
- Enright EF, Gahan CG, Joyce SA, Griffin BTJTYjob, and medicine (2016) Focus: microbiome: the impact of the gut microbiota on drug metabolism and clinical outcome. **89**:375.
- Erhardt A, Biburger M, Papadopoulos T, and Tiegs G (2007) IL-10, regulatory T cells, and Kupffer cells mediate tolerance in concanavalin A–induced liver injury in mice. *Hepatology* **45**:475-485.
- Fontana RJ, Hayashi PH, Gu J, Reddy KR, Barnhart H, Watkins PB, Serrano J, Lee WM, Chalasani N, Stolz A, Davern T, and Talwakar JA (2014) Idiosyncratic Drug-Induced Liver Injury Is Associated With Substantial Morbidity and Mortality Within 6 Months From Onset. *Gastroenterology* **147**:96-108.e104.
- Fountain FF, Tolley E, Chrisman CR, and Self TH (2005) Isoniazid Hepatotoxicity Associated With Treatment of Latent Tuberculosis Infection: A 7-Year Evaluation From a Public Health Tuberculosis Clinic. *CHEST* **128**:116-123.
- Fromenty B (2017) Role of nonalcoholic fatty liver disease as risk factor for drug-induced hepatotoxicity. *J Clin Transl Res* **3**.
- Fujimoto M, Uemura M, Nakatani Y, Tsujita S, Hoppo K, Tamagawa T, Kitano H, Kikukawa M, Ann T, Ishii YJAC, and Research E (2000) Plasma endotoxin and serum cytokine levels in patients with alcoholic hepatitis: relation to severity of liver disturbance. **24**:48S-54S.
- Funk C, Ponelle C, Scheuermann G, and Pantze MJMp (2001) Cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity: in

- vivo and in vitro interaction at the canalicular bile salt export pump (Bsep) in the rat. **59**:627-635.
- Ganey PE, Luyendyk JP, Maddox JF, and Roth RA (2004) Adverse hepatic drug reactions: inflammatory episodes as consequence and contributor. *Chemico-Biological Interactions* **150**:35-51.
- Gaude G, Chaudhury A, and Hattiholi J (2015) Drug-induced hepatitis and the risk factors for liver injury in pulmonary tuberculosis patients. **4**:238-243.
- Ghosh SS, Wang J, Yannie PJ, and Ghosh S (2020) Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. *Journal of the Endocrine Society* **4**.
- Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim M-S, Kim KH, Shneider BL, Picarsic JL, Jacobson TA, Zhang J, He W, Liu P, Knisely AS, Finegold MJ, Muzny DM, Boerwinkle E, Lupski JR, Plon SE, Gibbs RA, Eng CM, Yang Y, Washington GC, Porteus MH, Berquist WE, Kambham N, Singh RJ, Xia F, Enns GM, and Moore DD (2016) Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. *Nature Communications* **7**:10713.
- Gong S, Lan T, Zeng L, Luo H, Yang X, Li N, Chen X, Liu Z, Li R, and Win SJJoh (2018) Gut microbiota mediates diurnal variation of acetaminophen induced acute liver injury in mice. **69**:51-59.
- Gonzalez FJ, Fang ZZ, and Ma X (2015) Transgenic mice and metabolomics for study of hepatic xenobiotic metabolism and toxicity. *Expert Opin Drug Metab Toxicol* **11**:869-881.
- Goodwin B, Hodgson E, and Liddle CJMp (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. **56**:1329-1339.

- Gopaul S, Farrell K, and Abbott F (2003) Effects of Age and Polytherapy, Risk Factors of Valproic Acid (VPA) Hepatotoxicity, on the Excretion of Thiol Conjugates of (E)-2,4-diene VPA in People with Epilepsy Taking VPA. *Epilepsia* **44**:322-328.
- Gorski JC, Huang SM, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA, Desai M, Miller M, Hall SDJCP, and Therapeutics (2004) The effect of echinacea (Echinacea purpurea root) on cytochrome P450 activity in vivo. **75**:89-100.
- Greene SL, Dargan PI, O'connor N, Jones AL, and Kerins MJTAjoem (2003) Multiple toxicity from 3, 4-methylenedioxymethamphetamine (“ecstasy”). **21**:121-124.
- Haas DW, Koletar SL, Laughlin L, Kendall MA, Suckow C, Gerber JG, Zolopa AR, Bertz R, Child MJ, and Hosey LJJoaid (2009) Hepatotoxicity and gastrointestinal intolerance when healthy volunteers taking rifampin add twice-daily atazanavir and ritonavir. **50**:290.
- Haenisch S, Zimmermann U, Dazert E, Wruck CJ, Dazert P, Siegmund S, Kroemer HK, Warzok RW, and Cascorbi I (2007) Influence of polymorphisms of ABCB1 and ABCC2 on mRNA and protein expression in normal and cancerous kidney cortex. *The Pharmacogenomics Journal* **7**:56-65.
- Hamesch K, Borkham-Kamphorst E, Strnad P, and Weiskirchen R (2015) Lipopolysaccharide-induced inflammatory liver injury in mice. *Laboratory Animals* **49**:37-46.
- Hinson JA, Forkert P-GJCjop, and pharmacology (1995) Phase II enzymes and bioactivation. **73**:1407-1413.

- Hogaboam CM, Simpson KJ, Chensue SW, Steinhauser ML, Lukacs NW, Gauldie J, Strieter RM, and Kunkel SL (1999) Macrophage inflammatory protein-2 gene therapy attenuates adenovirus- and acetaminophen-mediated hepatic injury. *Gene Therapy* **6**:573-584.
- Hoofnagle JH and Björnsson ES (2019) Drug-Induced Liver Injury — Types and Phenotypes. *New England Journal of Medicine* **381**:264-273.
- Huang Y-S, Chern H-D, Su W-J, Wu J-C, Lai S-L, Yang S-Y, Chang F-Y, and Lee S-DJH (2002) Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. **35**:883-889.
- Huang Y-S, Su W-J, Huang Y-H, Chen C-Y, Chang F-Y, Lin H-C, and Lee S-D (2007) Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. *Journal of Hepatology* **47**:128-134.
- Huang Y, Furuno M, Arakawa T, Takizawa S, de Hoon M, Suzuki H, and Arner E (2019) A framework for identification of on- and off-target transcriptional responses to drug treatment. *Scientific Reports* **9**:17603.
- Hunt PW (2012) HIV and Inflammation: Mechanisms and Consequences. *Current HIV/AIDS Reports* **9**:139-147.
- Jackson JP, Freeman KM, St. Claire RL, Black CB, and Brouwer KR (2018) Cholestatic Drug Induced Liver Injury: A Function of Bile Salt Export Pump Inhibition and Farnesoid X Receptor Antagonism. *Applied In Vitro Toxicology* **4**:265-279.
- Jaeschke H, McGill MR, and Ramachandran A (2012) Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. *Drug metabolism reviews* **44**:88-106.

- Jakoby WBJMie (1985) [61] Glutathione transferases: An overview. **113**:495-499.
- James LP, Lamps LW, McCullough S, and Hinson JA (2003) Interleukin 6 and hepatocyte regeneration in acetaminophen toxicity in the mouse. *Biochemical and biophysical research communications* **309**:857-863.
- Kaitin KJTCftSoDDIR (2005) Drug safety withdrawals in the US not linked to speed of FDA approval. **7**.
- Kato T, Ito Y, Hosono K, Suzuki T, Tamaki H, Minamino T, Kato S, Sakagami H, Shibuya M, and Majima M (2011) Vascular Endothelial Growth Factor Receptor-1 Signaling Promotes Liver Repair through Restoration of Liver Microvasculature after Acetaminophen Hepatotoxicity. *Toxicological Sciences* **120**:218-229.
- Kepler D (2014) The Roles of MRP2, MRP3, OATP1B1, and OATP1B3 in Conjugated Hyperbilirubinemia. *Drug Metabolism and Disposition* **42**:561.
- Kim WS, Lee SS, Lee CM, Kim HJ, Ha CY, Kim HJ, Kim TH, Jung WT, Lee OJ, Hong JW, You HS, and Cho HC (2016) Hepatitis C and not Hepatitis B virus is a risk factor for anti-tuberculosis drug induced liver injury. *BMC Infectious Diseases* **16**:50.
- Koliaki C and Roden M (2013) Hepatic energy metabolism in human diabetes mellitus, obesity and non-alcoholic fatty liver disease. *Molecular and Cellular Endocrinology* **379**:35-42.
- Kostrubsky SE, Sinclair JF, Strom SC, Wood S, Urda E, Stolz DB, Wen YH, Kulkarni S, and Mutlib A (2005) Phenobarbital and Phenytoin Increased Acetaminophen Hepatotoxicity Due to Inhibition of UDP-Glucuronosyltransferases in Cultured Human Hepatocytes. *Toxicological Sciences* **87**:146-155.

- Kullak-Ublick GA, Andrade RJ, Merz M, End P, Benesic A, Gerbes AL, and Aithal GP (2017)
Drug-induced liver injury: recent advances in diagnosis and risk assessment. *Gut*
66:1154.
- Laine L, Goldkind L, Curtis SP, Connors LG, Yanqiong Z, and Cannon CP (2009) How
Common Is Diclofenac-Associated Liver Injury? Analysis of 17,289 Arthritis Patients in
a Long-Term Prospective Clinical Trial. *Official journal of the American College of*
Gastroenterology / ACG **104**.
- Lamb YN (2019) Pexidartinib: First Approval. *Drugs* **79**:1805-1812.
- Lauterburg B and Velez MEJG (1988) Glutathione deficiency in alcoholics: risk factor for
paracetamol hepatotoxicity. **29**:1153-1157.
- Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliewer SAJTJoci (1998)
The human orphan nuclear receptor PXR is activated by compounds that regulate
CYP3A4 gene expression and cause drug interactions. **102**:1016-1023.
- Li F, Lu J, Cheng J, Wang L, Matsubara T, Csanaky IL, Klaassen CD, Gonzalez FJ, and Ma X
(2013) Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid
co-therapy. *Nature Medicine* **19**:418-420.
- Li F, Lu J, and Ma X (2011) Profiling the reactive metabolites of xenobiotics using metabolomic
technologies. *Chem Res Toxicol* **24**:744-751.
- Li H, Chen Y, Zhang J, Chen X, Li Z, Liu B, and Zhang L (2019) Shikonin Attenuates
Acetaminophen-Induced Hepatotoxicity by Upregulation of Nrf2 through Akt/GSK3 β
Signaling. *Molecules* **24**.
- Li W and Kong ANJMC PicwtUoTMAcc (2009) Molecular mechanisms of Nrf2-mediated
antioxidant response. **48**:91-104.

- Lilja JJ, Kivistö KT, Neuvonen PJJCP, and Therapeutics (1999) Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. **66**:118-127.
- Lin H-S, Cheng C-W, Lin M-S, Chou Y-L, Chang P-J, Lin J-C, and Ye J-JCIIA (2016) The clinical outcomes of oldest old patients with tuberculosis treated by regimens containing rifampicin, isoniazid, and pyrazinamide. **11**:299.
- Lu J, Jones AD, Harkema JR, Roth RA, and Ganey PE (2012) Amiodarone Exposure During Modest Inflammation Induces Idiosyncrasy-like Liver Injury in Rats: Role of Tumor Necrosis Factor-alpha. *Toxicological Sciences* **125**:126-133.
- Lucena MI, Andrade RJ, Kaplowitz N, García-Cortes M, Fernández MC, Romero-Gomez M, Bruguera M, Hallal H, Robles-Diaz M, and Rodriguez-González JFJH (2009) Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. **49**:2001-2009.
- Luyendyk JP, Maddox JF, Cosma GN, Ganey PE, Cockerell GL, and Roth RA (2003) Ranitidine Treatment during a Modest Inflammatory Response Precipitates Idiosyncrasy-Like Liver Injury in Rats. *Journal of Pharmacology and Experimental Therapeutics* **307**:9.
- Lynch T and Price ALJAfp (2007) The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. **76**:391-396.
- Mach J, Huizer-Pajkos A, Mitchell SJ, McKenzie C, Phillips L, Kane A, Jones B, de Cabo R, Cogger V, Le Couteur DGJF, and pharmacology c (2016) The effect of ageing on isoniazid pharmacokinetics and hepatotoxicity in Fischer 344 rats. **30**:23-34.
- Mak A and Uetrecht J (2017) Immune mechanisms of idiosyncratic drug-induced liver injury. *J Clin Transl Res* **3**:145-156.

- Makhlouf HA, Helmy A, Fawzy E, El-Attar M, and Rashed HAGJHi (2008) A prospective study of antituberculous drug-induced hepatotoxicity in an area endemic for liver diseases. **2**:353-360.
- Matsumaru K, Ji C, and Kaplowitz N (2003) Mechanisms for sensitization to TNF-induced apoptosis by acute glutathione depletion in murine hepatocytes. *Hepatology* **37**:1425-1434.
- Mehendale HM (2005) Tissue Repair: An Important Determinant of Final Outcome of Toxicant-Induced Injury. *Toxicologic Pathology* **33**:41-51.
- Mehta SJDp and therapeutics (1990) Malnutrition and drugs: clinical implications. **15**:159-165.
- Meibohm B, Beierle I, and Derendorf H (2002) How Important Are Gender Differences in Pharmacokinetics? *Clinical Pharmacokinetics* **41**:329-342.
- Meier Y, Zodan T, Lang C, Zimmermann R, Kullak-Ublick GA, Meier PJ, Stieger B, and Pauli-Magnus CJWjogW (2008) Increased susceptibility for intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis in carriers of the 1331T> C polymorphism in the bile salt export pump. **14**:38.
- Metushi IG, Hayes MA, and Uetrecht J (2015) Treatment of PD-1^{-/-} mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. *Hepatology* **61**:1332-1342.
- Michalopoulos GK (2007) Liver regeneration. *J Cell Physiol* **213**:286-300.
- Michalopoulos GK and Khan Z (2005) Liver regeneration, growth factors, and amphiregulin. *Gastroenterology* **128**:503-506.
- Michalopoulos GKJTAjop (2010) Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. **176**:2-13.

- Minerali E, Foil DH, Zorn KM, Lane TR, and Ekins S (2020) Comparing Machine Learning Algorithms for Predicting Drug-Induced Liver Injury (DILI). *Mol Pharm* **17**:2628-2637.
- Mizutani T, Murakami M, Shirai M, Tanaka M, and Nakanishi KJJoat (1999) Metabolism-dependent hepatotoxicity of methimazole in mice depleted of glutathione. **19**:193-198.
- Mizutani T, Yoshida K, Murakami M, Shirai M, and Kawazoe SJCrit (2000) Evidence for the involvement of N-methylthiourea, a ring cleavage metabolite, in the hepatotoxicity of methimazole in glutathione-depleted mice: structure– toxicity and metabolic studies. **13**:170-176.
- Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet PJAC, and Research E (2009) Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. **33**:1836-1846.
- Nicoletti P, Aithal GP, Chamberlain TC, Coulthard S, Alshabeeb M, Grove JI, Andrade RJ, Bjornsson E, Dillon JF, Hallberg P, Lucena MI, Maitland-van der Zee AH, Martin JH, Molokhia M, Pirmohamed M, Wadelius M, Shen Y, Nelson MR, Daly AK, and the International Drug-Induced Liver Injury C (2019) Drug-Induced Liver Injury due to Flucloxacillin: Relevance of Multiple Human Leukocyte Antigen Alleles. *Clinical Pharmacology & Therapeutics* **106**:245-253.
- Nicoletti P, Devarbhavi H, Goel A, Venkatesan R, Eapen CE, Grove JI, Zafer S, Bjornsson E, Lucena MI, Andrade RJ, Pirmohamed M, Wadelius M, Larrey D, Maitland-van der Zee A-H, Ibanez L, Watkins PB, Daly AK, and Aithal GP (2021) Genetic Risk Factors in Drug-Induced Liver Injury Due to Isoniazid-Containing Antituberculosis Drug Regimens. *Clinical Pharmacology & Therapeutics* **109**:1125-1135.

- Nijland HM, Rafaëlla F, Rongen GA, van Uden P, van Crevel R, Boeree MJ, Aarnoutse RE, Koopmans PP, and Burger DMJA (2008) High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. *22*:931-935.
- Niu M-W and Chen P (2020) Gut microbiota and drug-induced liver injury: an update. *Chinese Medical Journal* **133**.
- Oben JA and Diehl AM (2004) Sympathetic nervous system regulation of liver repair. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* **280A**:874-883.
- Okada R, Maeda K, Nishiyama T, Aoyama S, Tozuka Z, Hiratsuka A, Ikeda T, Kusuhara H, and Sugiyama Y (2011) Involvement of Different Human Glutathione Transferase Isoforms in the Glutathione Conjugation of Reactive Metabolites of Troglitazone. *Drug Metabolism and Disposition* **39**:2290.
- Paananen J and Fortino V (2020) An omics perspective on drug target discovery platforms. *Brief Bioinform* **21**:1937-1953.
- Pais R, Rusu E, and Ratzu V (2014) The Impact of Obesity and Metabolic Syndrome on Chronic Hepatitis B and Drug-Induced Liver Disease. *Clinics in Liver Disease* **18**:165-178.
- Papastefanou VP, Bozas E, Mykoniatis MG, Grypioti A, Garyfallidis S, Bartsocas CS, and Nicolopoulou-Stamati P (2007) VEGF isoforms and receptors expression throughout acute acetaminophen-induced liver injury and regeneration. *Archives of Toxicology* **81**:729-741.

- Pateria P, de Boer B, and MacQuillan G (2013) Liver abnormalities in drug and substance abusers. *Best Practice & Research Clinical Gastroenterology* **27**:577-596.
- Pignataro P, da Silva Rocha A, Nery JAC, Miranda A, Sales AM, Ferreira H, Valentim V, and Suffys PN (2004) Leprosy and AIDS: two cases of increasing inflammatory reactions at the start of highly active antiretroviral therapy. *European Journal of Clinical Microbiology and Infectious Diseases* **23**:408-411.
- Quemard A, Lacave C, and Laneelle G (1991) Isoniazid inhibition of mycolic acid synthesis by cell extracts of sensitive and resistant strains of *Mycobacterium aurum*. *Antimicrob Agents Chemother* **35**:1035-1039.
- Ray PD, Huang B-W, and Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* **24**:981-990.
- Renjifo B, van Wyk J, Salem AH, Bow D, Ng J, and Norton MJAr (2015) Pharmacokinetic enhancement in HIV antiretroviral therapy: a comparison of ritonavir and cobicistat. **17**:37-46.
- Reuben A, Tillman H, Fontana RJ, Davern T, McGuire B, Stravitz RT, Durkalski V, Larson AM, Liou I, and Fix OJAoim (2016) Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. **164**:724-732.
- Roth AD and Lee M-Y (2017) Idiosyncratic Drug-Induced Liver Injury (IDILI): Potential Mechanisms and Predictive Assays. *BioMed Research International* **2017**:9176937.
- Rudmann DG (2012) On-target and Off-target-based Toxicologic Effects. *Toxicologic Pathology* **41**:310-314.
- Ruffmann R and Wendel A (1991) GSH rescue by N-acetylcysteine. *Klin Wochenschr* **69**:857-862.

- Russo MW, Galanko JA, Shrestha R, Fried MW, and Watkins PJLT (2004) Liver transplantation for acute liver failure from drug induced liver injury in the United States. **10**:1018-1023.
- Sachar M, Li F, Liu K, Wang P, Lu J, and Ma X (2016) Chronic Treatment with Isoniazid Causes Protoporphyrin IX Accumulation in Mouse Liver. *Chem Res Toxicol* **29**:1293-1297.
- Salden HJ and Bas BM (1994) Endotoxin binding to platelets in blood from patients with a sepsis syndrome. *Clinical Chemistry* **40**:1575-1579.
- Sandhu N and Navarro V (2020) Drug-Induced Liver Injury in GI Practice. *Hepatology Communications* **4**:631-645.
- Schenker S, Martin RR, and Hoyumpa AMJJoh (1999) Antecedent liver disease and drug toxicity. **31**:1088-1097.
- Schmidt LE and Dalhoff K (2003) The impact of current tobacco use on the outcome of paracetamol poisoning. *Alimentary Pharmacology & Therapeutics* **18**:979-985.
- Schmidt LE, Dalhoff K, and Poulsen HE (2002) Acute versus chronic alcohol consumption in acetaminophen-induced hepatotoxicity. *Hepatology* **35**:876-882.
- Schmitt C, Riek M, Winters K, Schutz M, and Grange SJAodi (2009) Unexpected hepatotoxicity of rifampin and saquinavir/ritonavir in healthy male volunteers. **2**:8-16.
- Schmucker DL, Sanchez HJCg, and research g (2011) Liver regeneration and aging: a current perspective. **2011**.
- Schroder K, Sagulenko V, Zamoshnikova A, Richards AA, Cridland JA, Irvine KM, Stacey KJ, and Sweet MJ (2012) Acute lipopolysaccharide priming boosts inflammasome activation independently of inflammasome sensor induction. *Immunobiology* **217**:1325-1329.

- Schulz W and Zillig W (1981) Rifampicin inhibition of RNA synthesis by destabilisation of DNA-RNA polymerase-oligonucleotide-complexes. *Nucleic Acids Res* **9**:6889-6906.
- Schwartz JB (2003) The Influence of Sex on Pharmacokinetics. *Clinical Pharmacokinetics* **42**:107-121.
- Seo KW, Park M, Kim JG, Kim TW, and Kim HJ (2000) Effects of benzothiazole on the xenobiotic metabolizing enzymes and metabolism of acetaminophen. *Journal of Applied Toxicology* **20**:427-430.
- Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, Lenoir C, Lemoine A, and Hillon P (2002) Incidence of drug-induced hepatic injuries: a French population-based study. *Hepatology* **36**:451-455.
- Shaw PJ, Ganey PE, and Roth RA (2009) Trovafloxacin Enhances the Inflammatory Response to a Gram-Negative or a Gram-Positive Bacterial Stimulus, Resulting in Neutrophil-Dependent Liver Injury in Mice. *Journal of Pharmacology and Experimental Therapeutics* **330**:72.
- Shaw PJ, Ganey PE, and Roth RA (2010) Idiosyncratic Drug-Induced Liver Injury and the Role of Inflammatory Stress with an Emphasis on an Animal Model of Trovafloxacin Hepatotoxicity. *Toxicological Sciences* **118**:7-18.
- Shaw PJ, Hopfensperger MJ, Ganey PE, and Roth RA (2007) Lipopolysaccharide and Trovafloxacin Coexposure in Mice Causes Idiosyncrasy-Like Liver Injury Dependent on Tumor Necrosis Factor-Alpha. *Toxicological Sciences* **100**:259-266.
- Shehu AI, Lu J, Wang P, Zhu J, Wang Y, Yang D, McMahon D, Xie W, Gonzalez FJ, and Ma X (2019) Pregnane X receptor activation potentiates ritonavir hepatotoxicity. *J Clin Invest* **129**:2898-2903.

- Shehu AI, Zhu J, Li J, Lu J, McMahon D, Xie W, Gonzalez FJ, and Ma X (2021) Targeting Xenobiotic Nuclear Receptors PXR and CAR to Prevent Cobicistat Hepatotoxicity. *Toxicological Sciences* **181**:58-67.
- Siegers CP (1978) Antidotal effects of dimethyl sulphoxide against paracetamol-, bromobenzene-, and thioacetamide-induced hepatotoxicity. *J Pharm Pharmacol* **30**:375-377.
- Silva MO, Roth D, Reddy KR, Fernandez JA, Albores-Saavedra J, and Schiff ERJoh (1991) Hepatic dysfunction accompanying acute cocaine intoxication. **12**:312-315.
- Simon T, Becquemont L, Mary-Krause M, de Waziers I, Beaune P, Funck-Brentano C, and Jaillon P (2000) Combined glutathione-S-transferase M1 and T1 genetic polymorphism and tacrine hepatotoxicity. *Clinical Pharmacology & Therapeutics* **67**:432-437.
- Singer JB, Lewitzky S, Leroy E, Yang F, Zhao X, Klickstein L, Wright TM, Meyer J, and Paulding CA (2010) A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nature Genetics* **42**:711-714.
- Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, and Basit AWJ (2008) The gastrointestinal microbiota as a site for the biotransformation of drugs. **363**:1-25.
- Speerhas R (1995) Drug metabolism in malnutrition and obesity: clinical concerns. *Cleveland Clinic Journal of Medicine* **62**:73.
- Spraggs CF, Budde LR, Briley LP, Bing N, Cox CJ, King KS, Whittaker JC, Mooser VE, Preston AJ, Stein SH, and Cardon LR (2011) HLA-DQA1*02:01 Is a Major Risk Factor for Lapatinib-Induced Hepatotoxicity in Women With Advanced Breast Cancer. *Journal of Clinical Oncology* **29**:667-673.

Steele MA, Burk RF, and DesPrez RMJC (1991) Toxic hepatitis with isoniazid and rifampin: a meta-analysis. **99**:465-471.

Stine JG and Chalasani NPJCild (2017) Drug hepatotoxicity: environmental factors. **21**:103-113.

Strähle U and Grabher C (2010) The zebrafish embryo as a model for assessing off-target drug effects. *Disease Models & Mechanisms* **3**:689-692.

Suzuki A, Yuen N, Walsh J, Papay J, Hunt CM, and Diehl AM (2009) Co-mediations That Modulate Liver Injury and Repair Influence Clinical Outcome of Acetaminophen-Associated Liver Injury. *Clinical Gastroenterology and Hepatology* **7**:882-888.

Sznol M, Ferrucci PF, Hogg D, Atkins MB, Wolter P, Guidoboni M, Lebbé C, Kirkwood JM, Schachter J, Daniels GA, Hassel J, Cebon J, Gerritsen W, Atkinson V, Thomas L, McCaffrey J, Power D, Walker D, Bhore R, Jiang J, Hodi FS, and Wolchok JD (2017) Pooled Analysis Safety Profile of Nivolumab and Ipilimumab Combination Therapy in Patients With Advanced Melanoma. *Journal of Clinical Oncology* **35**:3815-3822.

Tamai S, Iguchi T, Niino N, Mikamoto K, Sakurai K, Sayama A, Shimoda H, Takasaki W, and Mori K (2017) A monkey model of acetaminophen-induced hepatotoxicity; phenotypic similarity to human. *The Journal of Toxicological Sciences* **42**:73-84.

Tap WD, Gelderblom H, Palmerini E, Desai J, Bauer S, Blay J-Y, Alcindor T, Ganjoo K, Martín-Broto J, Ryan CW, Thomas DM, Peterfy C, Healey JH, van de Sande M, Gelhorn HL, Shuster DE, Wang Q, Yver A, Hsu HH, Lin PS, Tong-Starksen S, Stacchiotti S, and Wagner AJ (2019) Pexidartinib versus placebo for advanced tenosynovial giant cell tumour (ENLIVEN): a randomised phase 3 trial. *The Lancet* **394**:478-487.

- Telkoparan-Akillilar P, Suzen S, and Saso L (2019) Pharmacological Applications of Nrf2 Inhibitors as Potential Antineoplastic Drugs. *Int J Mol Sci* **20**.
- Thakur V, McMullen MR, Pritchard MT, Nagy LEJJog, and hepatology (2007) Regulation of macrophage activation in alcoholic liver disease. **22**:S53-S56.
- Townsend DM and Tew KD (2003) The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene* **22**:7369-7375.
- Toyoda Y, Miyashita T, Endo S, Tsuneyama K, Fukami T, Nakajima M, and Yokoi T (2011) Estradiol and progesterone modulate halothane-induced liver injury in mice. *Toxicology Letters* **204**:17-24.
- Uetrecht J (2007) Idiosyncratic Drug Reactions: Current Understanding. *Annual Review of Pharmacology and Toxicology* **47**:513-539.
- Umbaugh DS and Jaeschke H (2021) Extracellular vesicles: Roles and applications in drug-induced liver injury. *Adv Clin Chem* **102**:63-125.
- Utzeri E and Usai PJWjog (2017) Role of non-steroidal anti-inflammatory drugs on intestinal permeability and nonalcoholic fatty liver disease. **23**:3954.
- Vall A, Sabnis Y, Shi J, Class R, Hochreiter S, and Klambauer G (2021) The Promise of AI for DILI Prediction. *Frontiers in Artificial Intelligence* **4**.
- Villanueva-Paz M, Morán L, López-Alcántara N, Freixo C, Andrade RJ, Lucena MI, and Cubero FJ (2021) Oxidative Stress in Drug-Induced Liver Injury (DILI): From Mechanisms to Biomarkers for Use in Clinical Practice. *Antioxidants* **10**.
- Wada T, Ueda M, Abe K, Kobari T, Yamazaki H, Nakata J, Ikemoto I, Ohishi Y, and Aizawa YJHkAuJ (1999) Risk factor of liver disorders caused by flutamide--statistical analysis using multivariate logistic regression analysis. **45**:521-526.

- Wang P, Shehu AI, and Ma X (2017) The Opportunities of Metabolomics in Drug Safety Evaluation. *Curr Pharmacol Rep* **3**:10-15.
- Wang Z, Xu G, Zhan X, Liu Y, Gao Y, Chen N, Guo Y, Li R, He T, Song X, Niu M, Wang J, Bai Z, and Xiao X (2019) Carbamazepine promotes specific stimuli-induced NLRP3 inflammasome activation and causes idiosyncratic liver injury in mice. *Arch Toxicol* **93**:3585-3599.
- Wanwimolruk S and Prachayasittikul VJEj (2014) Cytochrome P450 enzyme mediated herbal drug interactions (Part 1). **13**:347.
- Waring JF, Liguori MJ, Luyendyk JP, Maddox JF, Ganey PE, Stachlewitz RF, North C, Blomme EAG, and Roth RA (2006) Microarray Analysis of Lipopolysaccharide Potentiation of Trovafloxacin-Induced Liver Injury in Rats Suggests a Role for Proinflammatory Chemokines and Neutrophils. *Journal of Pharmacology and Experimental Therapeutics* **316**:1080.
- Wassenaar TM, Zimmermann KJEJoM, and Immunology (2018) Lipopolysaccharides in food, food supplements, and probiotics: should we be worried? **8**:63-69.
- Watkins PB and Seeff LB (2006) Drug-induced liver injury: Summary of a single topic clinical research conference. *Hepatology* **43**:618-631.
- Wree A, Eguchi A, McGeough MD, Pena CA, Johnson CD, Canbay A, Hoffman HM, and Feldstein AE (2014) NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. *Hepatology* **59**:898-910.
- Wu L, Chen Y, Liu H, Zhan Z, Liang Z, Zhang T, Cai Z, Ye L, Liu M, Zhao J, Liu S, and Tang L (2018) Emodin-induced hepatotoxicity was exacerbated by probenecid through inhibiting UGTs and MRP2. *Toxicology and Applied Pharmacology* **359**:91-101.

- Xiao Dong S, Zhi Ping Z, Zhong Xiao W, Chong Shu C, Bartoli A, Gatti G, D'Urso S, and Perucca E (1998) Effect of Active and Passive Cigarette Smoking on CYP1A2-Mediated Phenacetin Disposition in Chinese Subjects. *Therapeutic Drug Monitoring* **20**.
- Xu L, Liu H, Murray BP, Callebaut C, Lee MS, Hong A, Strickley RG, Tsai LK, Stray KM, and Wang YJAmcl (2010) Cobicistat (GS-9350): a potent and selective inhibitor of human CYP3A as a novel pharmacoenhancer. **1**:209-213.
- Yan T, Wang H, Zhao M, Yagai T, Chai Y, Krausz KW, Xie C, Cheng X, Zhang J, Che Y, Li F, Wu Y, Brocker CN, Gonzalez FJ, Wang G, and Hao H (2016) Glycyrrhizin Protects against Acetaminophen-Induced Acute Liver Injury via Alleviating Tumor Necrosis Factor α -Mediated Apoptosis. *Drug Metab Dispos* **44**:720.
- Yan T, Yan N, Wang H, Yagai T, Luo Y, Takahashi S, Zhao M, Krausz KW, Wang G, Hao H, and Gonzalez FJ (2021) FXR-Deoxycholic Acid-TNF- α Axis Modulates Acetaminophen-Induced Hepatotoxicity. *Toxicol Sci* **181**:273-284.
- Yimer G, Amogne W, Habtewold A, Makonnen E, Ueda N, Suda A, Worku A, Haefeli WE, Burhenne J, Aderaye G, Lindquist L, and Aklillu E (2012) High plasma efavirenz level and *CYP2B6**6 are associated with efavirenz-based HAART-induced liver injury in the treatment of naïve HIV patients from Ethiopia: a prospective cohort study. *The Pharmacogenomics Journal* **12**:499-506.
- Yip LY, Aw CC, Lee SH, Hong YS, Ku HC, Xu WH, Chan JMX, Cheong EJY, Chng KR, Ng AHQ, Nagarajan N, Mahendran R, Lee YK, Browne ER, and Chan ECY (2018) The liver–gut microbiota axis modulates hepatotoxicity of tacrine in the rat. *Hepatology* **67**:282-295.

- Zaverucha-do-Valle C, Monteiro SP, El-Jaick KB, Rosadas LA, Costa MJM, Quintana MSB, and de Castro L (2014) The role of cigarette smoking and liver enzymes polymorphisms in anti-tuberculosis drug-induced hepatotoxicity in Brazilian patients. *Tuberculosis* **94**:299-305.
- Zhu J, Lu J, Tung HC, Liu K, Li J, Grant DM, Xie W, and Ma X (2020) Cell Type-Specific Roles of CD38 in the Interactions of Isoniazid with NAD(+) in the Liver. *Drug Metab Dispos* **48**:1372-1379.
- Zimmerman HJ and Maddrey WC (1995) Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: Analysis of instances of therapeutic misadventure. *Hepatology* **22**:767-773.
- Zou W, Beggs KM, Sparkenbaugh EM, Jones AD, Younis HS, Roth RA, and Ganey PE (2009a) Sulindac Metabolism and Synergy with Tumor Necrosis Factor- α in a Drug-Inflammation Interaction Model of Idiosyncratic Liver Injury. *Journal of Pharmacology and Experimental Therapeutics* **331**:114.
- Zou W, Devi SS, Sparkenbaugh E, Younis HS, Roth RA, and Ganey PE (2009b) Hepatotoxic Interaction of Sulindac with Lipopolysaccharide: Role of the Hemostatic System. *Toxicological Sciences* **108**:184-193.

Footnotes

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Figure legends

Figure 1. Schematic representation for the off-targets of drugs in hepatocytes. For most drugs, the therapeutic targets are well-defined, whereas the off-targets are understudied and largely unknown. We predict that a victim drug delivers the first hit to hepatocytes through off-targets including enzymes, receptors, and transporters and co-factors potentiate DILI by providing the second hit to the pathways that are related to the off-targets of the victim drug.

Figure 2. Schematic representation for the roles of co-factors in DILI. Three steps are needed in DILI: (1) exposure of drug and its metabolites to the liver; (2) interactions of drug and its metabolites with molecular targets in the liver; and (3) inappropriate cell responses including cell defense, adaptation, and tissue repair. Specific co-factors can potentiate DILI by directly targeting a molecule that is involved in the three steps of liver injury. General co-factors can potentiate DILI by affecting multiple molecules in the process of liver injury.

Figure 1

Figure 2