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Editorial

Alcohol and drug-induced liver injury: Metabolism, mechanisms, pathogenesis and potential therapies☆

The liver is one of the major metabolic organs in the body, playing a critical role in the metabolism of carbohydrates, proteins, amino acids, lipids, drugs and xenobiotics. As a result, the liver often becomes the target of drug and xenobiotics-induced damage. Currently, the most common cause for acute liver failure in the United States and multiple European countries is drug-induced liver injury (DILI). This is one of the leading causes for halting drug development and removing drugs from the consumer market.

In Asia, the incidence of liver injury induced by using herbal or traditional medicine is also on the rise. In addition to DILI, alcohol abuse and alcoholic liver disease (ALD), is another major health problem worldwide that claimed more than 3 million lives in 2012. Liver cirrhosis was the 12th leading cause of death in the United States in 2015, 49.5% of which was estimated to be attributed to ALD according to a report published by the National Institute on Alcohol Abuse and Alcoholism (NIAAA).[1] The pathogenesis of ALD begins with alcoholic steatosis to alcoholic hepatitis (AH), liver fibrosis, cirrhosis and finally hepatocellular carcinoma (HCC).[2–4] Unfortunately, there are no available treatments for ALD.

In this special issue, multiple review articles and research papers were gathered to provide a cumulative understanding on the current understanding of hepatic drug metabolism, DILI and steatosis, steatotic ischemia-reperfusion injury and ALD as well as potential serum biomarkers and role of long non-coding RNA (lncRNA) in HCC.

The liver is a central organ that is enriched with drug metabolizing enzymes, which play a critical role in the metabolism, elimination and detoxification of drugs and xenobiotics, including alcohol. These metabolism enzymes include phase I enzymes (mainly cytochrome P450 (CYP) oxidases), phase II reactions (conjugation enzymes) and phase III excretion (transporters). As a result, drugs and xenobiotics will be added with reactive and polar groups, which convert lipophilic drugs into hydrophilic products to be more readily excreted. These reactions help the body to detoxify the drugs/xenobiotics, although some metabolic intermediates have also caused toxic effects.

Among all the known human CYP enzymes, the CYP3A family enzymes are responsible for metabolizing almost half of the currently prescribed drugs. The review paper from Dr. Chen's group[5] elegantly summarizes CYP3A family enzymes and their substrate recognition and modulation by small molecules. The review article goes into in depth discussion on the structural perspectives of CYP3A, along with a detailed comparison of CYP3A to other human CYP enzymes. The information in this review paper may help to design specific CYP3A inhibitors that may affect drug-drug interactions and other important biological processes. In addition to CYP-mediated phase I metabolism, phase II enzyme also plays important roles in biotransformation of drugs/xenobiotics. There are many phase II metabolizing enzymes including uridine diphosphate (UDP)-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), N-acetyltransferases (NATs) and glutathione S-transferases. Dr. Xie's group[6] from the University of Pittsburgh provided novel insights on the role of nuclear hepatocyte nuclear factor 4alpha (HNF4α) in the regulation of cholesterol sulfotransferase 2B1b (SULT2B1b) and their impact on hepatic glucose homeostasis. HNF4α is a nuclear receptor transcription factor that binds to deoxyribonucleic acid (DNA) as a homodimer, which regulates the group of hepatic CYP genes and glucose, as well as lipid metabolism genes. There are 44 cytosolic SULTs; and the SULT2 family enzymes primarily add sulfonation on neutral steroids (SULT2A) and sterols (SULT2B). There are two isoforms of SULT2B1 hydroxysteroid sulfotransferase, SULT2B1b catalyzes the sulfonation of 3β-hydroxysteroid hormones and cholesterol, whereas SULT2B1a catalyzes pregnenolone sulfonation. Obesity and the transition from fasting to the fed state increased hepatic expression of SULT2B1b which then inhibits HNF4α, thereby decreasing glucose production and gluconeogenic gene expression. Interestingly, activation of HNF4α increases the expression of SULT2b1B, suggesting a negative feedback loop between SULT2B1b and HNF4α in the regulation of glucose homeostasis. Therefore, in addition to regulating drug metabolism, phase III enzymes, such as SULT2B1b, also plays an important role in regulating energy homeostasis, particularly gluconeogenesis.

Acetaminophen (APAP) overdose can cause severe hepatotoxicity and is one of the top causes of acute liver failure in the United States.[7] It is well known that therapeutic dose of APAP is safe as majority (85–90%) of APAP undergoes glucuronidation and sulfation and secretes to bile and plasm from the liver, whereas a small portion (15–10%) of APAP is metabolized by CYP2E1 to generate a reactive metabolite, *N*-acetyl-p-benzoquinone imine (NAPQI).[8] NAPQI further conjugates with liver glutathione (GSH) to be detoxified. However, overdose of APAP leads to the generation of excessive NAPQI that depletes hepatocellular GSH and further covalently binds to intracellular proteins to form APAP-adducts (APAP-AD). Some of the APAP-AD are on the mitochondria that leads to mitochondrial damage and subsequent necrosis and liver injury. It has been well accepted that mitochondrial damage is the key event resulting in APAP-induced necrosis and liver injury.[9] The liver is a unique organ of which damaged hepatocytes can be replaced by

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proliferating hepatocytes via liver regeneration. In the review article entitled "Mitochondrial damage and biogenesis in acetaminophen-induced liver injury", Jaeschke *et al.*[10] highlights recent progress on targeting mitochondrial and mitochondrial biogenesis for ameliorating APAP-induced liver injury. Activation of c-Jun *N*-terminal kinase (JNK) especially mitochondrial JNK translocation is associated with APAP-induced hepatocyte necrosis. Post-treatment with 4-methylpyrazole (4 MP) did not affect oxidative metabolism and protein adduct formation; however, it did significantly attenuate APAP-induced mitochondrial translocation of JNK and liver injury. Mitochondrial biogenesis is regulated at the transcription level by several transcription factors, including the nuclear respiratory factor (NRF) 1 & 2, and mitochondrial transcription factor A (Tfam), and transcription coactivator peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1α). Increased PGC1α by SRT1720 or recombinant fibroblast growth factor 21 (FGF21) attenuates APAP-induced liver injury by increasing mitochondrial biogenesis and liver regeneration. These data clearly indicate that targeting mitochondrial biogenesis may be a promising approach for treating APAP-induced liver injury.

In addition to regulating cell death, mitochondria also play critical roles in regulating lipid metabolism, as mitochondria are the major sites for fatty acid beta-oxidation. Since many drugs can cause mitochondrial dysfunction, it is not surprising that drugs including APAP, amiodarone, ibuprofen, linezolid, etc., all can lead to hepatic steatosis. However, some drugs can also induce steatosis independent of mitochondrial damage. Dr. Fromenty's review article[11] in this issue summarizes the role and mechanisms of drug induced steatosis.

There are two types of DILI, the first is intrinsic DILI, DILI in this category is predictable and caused by a known source, usually an overdose. (*e.g.,* APAP). The second type of DILI is idiosyncratic, which is considered unpredictable and can happen at what is considered a therapeutic dose. In the review article entitled "Hepatic macrophages in drug-induced liver injury", Shan and Ju[12] present the most current knowledge on the role of hepatic macrophages in DILI. Liver macrophages contain the resident macrophages (also known as Kupffer cells) and the infiltrating monocyte-derived macrophages. Increasing evidence suggests that hepatic macrophages play important roles in both intrinsic DILI and idiosyncratic DILI. Accumulating evidence suggests that Kupffer cells and infiltrating macrophages may promote liver regeneration to ameliorate DILI.

In Asian countries, the incidence of acute liver injury and liver failure due to the consumption of herbal teas or traditional medicine is on the rise and is becoming one of the major causes of acute live failure. Pyrrolizidine alkaloids (PAs) are the ester derivatives of necine base and necic acid, which are commonly found in more than 6000 plants which is found in high amounts in the Chinese medicine Tusanqi (Gynura segetum). Notably, hundreds of people in China have been developing hepatic sinusoidal obstruction syndrome (HSOS) after ingesting Tusanqi. PAs are predominantly metabolized in the liver by CYP3A, generating reactive metabolites dehydropyrrolizidine alkaloids (DHPAs), which is further hydrolyzed to dehydroretronecine (DHR) when it binds to cellular GSH to form GSH-conjugates. Once GSH is depleted, DHPAs and DHR bind to proteins to form pyrrole-protein adducts to initiate the hepatotoxicity likely by inducing mitochondrial fragmentation in hepatocytes.[13] In addition to damaging the parenchymal cells, PAs can also damage the non-parenchyma cells, such as the hepatic sinusoidal endothelial cells (HSECs). In a review article in this issue, Xu and her colleagues[14] have highlighted the current understanding of the hepatotoxicity induced by PAs.

ALD is a major chronic liver disease worldwide. One of the early major pathological features of ALD is hepatic steatosis, which is characterized by the accumulation of lipid droplets (LDs) in hepatocytes. LDs are intracellular organelles that store intracellular triglycerides (TG) and cholesteryl esters (CE), which are generally thought to be originated from endoplasmic reticulum (ER). The hydrophobic mixture of TG and CE within the ER lumen protrudes out of ER to form LD with the ER-derived delimiting phospholipid monolayer. Chronic alcohol consumption induces the accumulation of hepatic LDs, which may involve multiple mechanisms including increased uptake of circulating free fatty acids, increased *de novo* lipogenesis, decreased mitochondria beta-oxidation, and very low-density lipoprotein (VLDL) secretion as well as deceased autophagic degradation of LDs via impaired lipophagy.[15] Decreased lipophagy is likely due to the decreased hepatic lysosome numbers as chronic alcohol consumption may impair transcription factor EB (TFEB), a master regulator of lysosomal biogenesis.[16] In addition to lysosome-mediated LD catabolism, alcohol may also impair adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL)-mediated lipolysis in hepatocytes resulting in the accumulation of LDs. In a review article of this issue, Dr. Schulze and Ding[17] have summarized the dynamic changes of LD and the role of lysosome in the regulation of LD homeostasis after alcohol. Besides the autophagy-lysosomal pathway, ubiquitin proteasome system (UPS) is another cellular catabolism pathway in mammalian cells. Interestingly, both the autophagy and UPS pathways may connect with each other as decreased UPS activity can activate autophagy and vice versa, decreased autophagy may also lead to compensatory activation of autophagy.[18,19] Dr. Donohue *et al.*[20] have excellently discussed how alcohol consumption can impair both autophagy and UPS in the liver resulting in the accumulation of LDs and Mallory-Denk bodies and liver injury in the review article entitled "Lysosome and proteasome dysfunction in alcohol-induced liver injury".

The composition of dietary fat is known to play important roles in the pathogenesis of ALD. The review from Zirnheld *et al.*[21] highlights recent advances regarding the role of dietary fat, distinct fatty acids, and bioactive fatty acid metabolites in ALD. Both deleterious and beneficial effects of distinct dietary polyunsaturated fatty acids (PUFAs) in ALD have been reported in clinical ALD animal models. Dietary enrichment in the n-6 PUFA and linoleic acid resulted in exacerbation of liver injury in experimental ALD. In contrast, little is known of the effects of other PUFAs, specifically n-3 PUFAs on ALD. Future research is required to determine the role of dietary PUFAs and their bioactive metabolites in clinical ALD. Interestingly, different types of dietary fat can also alter the gut microbiome, which can influence individual susceptibility to ALD, and affect disease severity. Dr. Feng's group[22] comprehensively reviewed the role and mechanisms of gut microbiome in the pathogenesis of ALD. Microbiome dysbiosis can induce short chain fatty acid changes, alter bile acid metabolism, impair intestinal barrier function, and increase the release of bacterial and fungal products resulting in inflammation; all of which contribute to the pathogenesis of ALD. Strategies to target the microbiome, including dietary nutrient interference, herbal medicine, antibiotics, anti-fungal agents, probiotics, engineered bacterial therapy, fecal transplantation, oral hygiene and bacteriophage have become a potential strategy for the prevention and treatment of ALD.

For end-stage of liver diseases, including DILI and ALD, liver transplantation remains the most effective treatment option. However, the shortage of liver organ donors has led to more aggressive acceptance and usage of liver grafts from extended criteria donors, including using steatotic livers. The review from Sun *et al.*[23] highlights the role of necroptosis in ischemia/reperfusion injury of steatotic livers.

Necroptosis is a regulated non-apoptotic cell death that is mediated by the receptor-interacting protein1 (RIP1), RIP3 and its

downstream molecule mixed lineage kinase domain-like protein (MLKL). Necroptosis is morphologically similar to necrosis, which is activated when caspase-8 is inhibited leading to RIP1 and RIP3 heterodimerization resulting in the assembly of the necrosome. The necrosome then activates MLKL by increasing the phosphorylation of MLKL resulting in MLKL plasma membrane translocation and oligomerization, and the eventual plasma membrane rupture and necrosis. Both alcoholic and non-alcoholic fatty liver induce the expression and activation of necroptosis proteins including RIP3 and MLKL, and MLKL knockout mice were resistant to ischemia-reperfusion injury when these mice were fed either with a normal chow diet or a high-fat diet.[24] Since pharmacological inhibitors for RIP1 and RIP3 are available, targeting RIP1-RIP3-MLKL-mediated necroptosis may be promising to extend the use of steatotic liver for liver transplantation by improving ischemia-reperfusion liver injury.

As discussed above, some of the late stage of ALD patients may eventually develop HCC. HCC is currently the fifth most common malignant cancer worldwide, without successful treatment. Liver transplantation is still one of the most efficient therapies for small unresectable HCC with cirrhosis. In an original research paper of this special issue, Zeng *et al.*[25] investigated the prognostic potential of preoperative fibrinogen levels in HCC patients receiving liver transplantation. It was found that a preoperative serum fibrinogen level above 2.68 g/L was associated with tumor recurrence in patients after liver transplantation. It is possible that the pre-transplant plasma fibrinogen levels may be used for patient selection who would more benefit from liver transplantation. However, more future works are needed to confirm this because it was only from a single-centered retrospective study. LncRNAs are a type of RNAs longer than 200 bp that are not translated into proteins but have been implicated in regulation of gene transcription through the recruitment of chromatin-modifying enzymes. In a study of this issue, Deng *et al.*[26] identified RP11-307C12.11 using The Cancer Genome Atlas (TCGA) database and investigated its role *in vitro* cultured liver tumor cell lines and *in vivo* tumor xenograft models. They found that overexpression of RP11-307C12.11 promoted HCC cell growth both *in vitro* and *in vivo*. Mechanistically, RP11-307C12.11 increased expression of CCND1 and PDK1 likely by affecting microRNA (miR)-138 expression.

In summary, articles in this special issue summarize and discuss the current knowledge on drug metabolism enzymes that are important for both DILI and ALD. It also provides an update on the role of mitochondrial damage and innate immunity in DILI and how DILI leads to hepatic steatosis. Several novel aspects on the pathogenesis of ALD are mentioned, including LD biogenesis and catabolism, autophagy and UPS, dietary factors, microbiota and steatotic hepatic ischemia and reperfusion injury. Finally, potential clinical markers and treatment targets for HCC are also discussed in this issue. Due to the page limitation, genetic factors, which are also important for both DILI and ALD, are not covered in this special issue which may be an interesting topic for future issues.

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21 November 2019