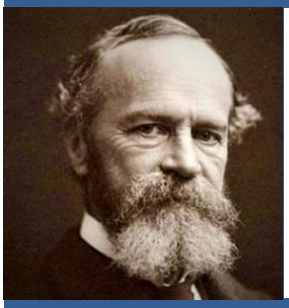
www.toxgurukul.org.in

ToxGurukul Foundation

TOXICOLOGY
NEWSLETTERS



"Is life worth living? It all depends on the liver"
-William James (1842-1910)

July, 2019

Hepatotoxicity

K.S. Rao[1], M.V.Sc., Ph.D., DABT ✉ and Shekar Chelur[2], M.V.Sc., DABT, DIBTP ✉
[1]Eurofins Advinus Limited, Bangalore and [2]Aurigene Discovery Technologies Ltd, Bangalore

1. Preamble

Over the last 20 years, it has become increasingly obvious that the liver is the most common target organ for toxicity which is adversely affected by an array of drugs and chemicals. Humans are exposed to these as medicines, industrial and environmental chemicals, food chemicals and a variety of naturally occurring substances. The level of exposure varies from minute quantities to very high doses. Exposure may be over a long period of time or limited to a single event, and it may be due to a single substance or to multiple chemicals. The circumstances of exposure may be an inadvertent, accidental, or intentional overdose or therapeutic necessity. Some chemicals cause an acute injury and others produce chronic liver changes that may lead to end-stage hepatic failure and in some cases hepatic malignancies.

The process of inventing drugs (products) and developing through various evaluation endpoints is complex, expensive, time-consuming and herculean task with 13.8% clinical success rate across all therapeutic indications (Wong et al., 2018). *Approximately 40% of total novel candidates developed are terminated due to non-clinical toxicology reasons* (Waring et al., 2015). Hence, the pharmaceutical industries try to detect toxic drug effects at the earliest to avoid the progress of such drug candidates into more expensive drug development stages. Liver safety assessment in preclinical species is mandatory in novel drug or chemical development. *Drug-induced hepatotoxicity is one of the most frequently cited reasons for refusal to approve drugs, drug withdrawal from the market, discontinuation.*

The liver plays a central role in processing dietary carbohydrates, lipids, amino acids, and vitamins; in the synthesis and turnover of most plasma proteins; and in the detoxification and biliary excretion of endogenous wastes and xenobiotic compounds. The liver also functions as an important organ of the innate immune system, integrated into the complex system of defense against foreign

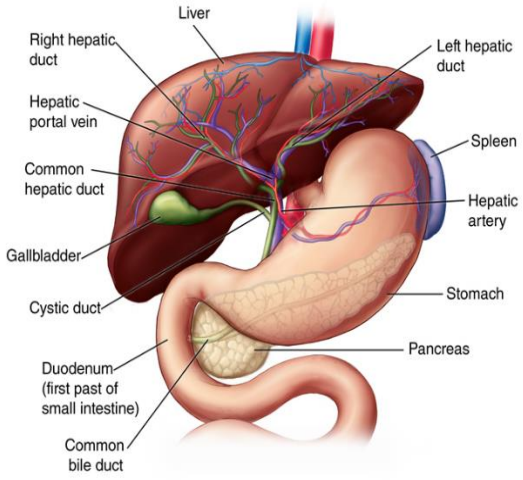
Editor
Alex Thomas
+91-974-545-0045
alexthomas.a@gmail.com

macromolecules. As such, hepatic disorders have far-reaching consequences, given the dependence of other organs on the metabolic function of the liver. As a result, Liver is considered the guardian of homeostasis, the epicenter of the body's metabolic capability, a massive filter detoxifying the portal blood, releasing cleansed blood to the systemic circulation, and a lymphoid organ protecting against infection. Owing to the liver's diverse functions and small mass in relation to the resting cardiac output that it handles, the liver is a target both for chemicals that are pharmacologically active and for toxic materials.

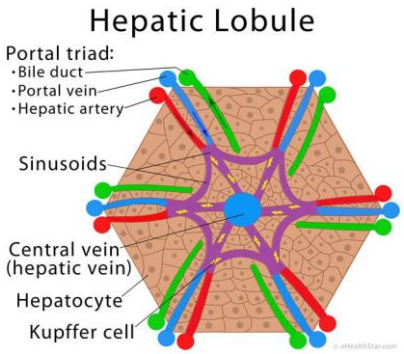
In this newsletter, we will cover the basics of liver, anatomy, physiology and metabolic role, biomarkers and few aspects of liver toxicity that are relevant to assessing the safety of chemicals. However, we will not make any attempt to cover hundreds of drugs and chemicals that cause liver toxicity. Also, it is not possible to cover various types of liver histopathology changes which are outside the scope of this newsletter.

2. Structure and Function of Liver

The liver consists of two main lobes and two minor lobes in humans. The lobes are made up of several segments and the segments are made up of a thousand lobules. The lobules are connected to small ducts that connect with larger ducts to ultimately form the common hepatic duct. the common hepatic duct. The common hepatic duct transports bile produced by the liver cells to the gallbladder and duodenum.



The liver is organized into lobules around terminal branches of the hepatic vein. Between the lobules are portal triads. Each triad consists of branches of a bile duct, portal vein, and hepatic artery.



The liver cells are separated from the sinusoids by a narrow space (space of Disse) that contains connective tissue and represents the scant interstitial compartment of the liver. Specialized cells of the macrophage system (Kupffer cells) are present in the sinusoids scattered among the endothelial cells.

The biliary system begins at the biliary canaliculi, which are small channels lined by the complex microvilli of surrounding liver cells. The biliary canaliculi form the intralobular bile ductules (canals of Hering), which drain into the bile ducts in the portal tract.

The liver regulates most chemical levels in the blood and excretes a product called bile. Bile released into the small intestines helps to break down fats, preparing them for further digestion and absorption. All of the blood leaving the stomach and intestines passes through the liver. The liver processes this blood and breaks down balances, and creates nutrients for the body to use. It also metabolized drugs in the blood into forms that are easier for the body to use. Many vital functions have been identified with the liver. Some of the more well-known functions include the following:

i. *Production of bile, which helps carry away waste and break down fats in the small intestine during digestion*
ii. *Production of certain proteins for blood plasma*
iii. *Production of cholesterol, phospholipids and special proteins to help carry fats through the body*
iv. *Store and release glucose as needed*
v. *Processing of hemoglobin for use of its iron content (the liver stores iron)*
vi. *Conversion of harmful ammonia to urea (urea is one of the end products of protein metabolism that is excreted in the urine)*
vii. *Detoxification, modification, and excretion of exogenous and endogenous substances including drugs and other harmful substances*
viii. *Regulating blood clotting through the production of clotting factors*
ix. *Resisting infections by producing immune factors and removing bacteria from the bloodstream*
x. *Clearance of bilirubin (if there is a buildup of bilirubin, the skin and eyes turn yellow)*

When the liver has broken down harmful substances, they are excreted into the bile or blood. Bile by-products enter the intestine and ultimately leave the body in the feces. There are considerable differences in anatomy, physiology and histology of liver in preclinical species including expression of drug metabolizing and other enzymes.

3. Liver an Organ for Drug Metabolism

The liver is the main metabolic organ in the body and is considered a viable defense against environmental toxicants and metabolic toxins. In general, all foreign compounds (xenobiotics) are potentially toxic. *In order to minimize the potential injury caused by these compounds, the liver is well equipped with metabolizing enzymes including Phase I and Phase II metabolizing enzymes as well as Phase III transporters.* The coordinated metabolism and transport of xenobiotics typically make the xenobiotic less toxic and more water soluble, thereby aiding its elimination from the body. Classically, the initial metabolic step is referred to as Phase I or the oxidation phase. This step is most frequently catalyzed by a cytochrome P450 (CYP). *CYPs derive their name from the fact that they exhibit maximal absorbance at 450 nm when bound to carbon monoxide*. CYPs are found in the highest abundance in the liver (specifically in hepatocytes) and to a lesser extent in the epithelium lining the gastrointestinal tract. They are typically found associated with the lipid membrane of the endoplasmic reticulum and mitochondrial outer membranes. *CYP1, CYP2, and CYP3 families are major enzymes mediating drug metabolisms and some P450s are highly inducible.* In general, Phase I CYP metabolism introduces a functional group to the xenobiotic, such as a hydroxyl group. Phase I metabolism may not be a detoxifying function by itself since the metabolite may actually be more reactive and toxic than the parent compound. Phase I metabolism often works in conjunction with subsequent Phase II metabolism to produce a more water soluble and less toxic compound.

The functional group added by Phase I metabolism is often the target of Phase II conjugation, typically making the metabolite more water soluble and less toxic. During this phase, the compound may undergo glucuronidation, sulfation, conjugation with glutathione, methylation, N-acetylation, or conjugation with amino acids. Typically, these modifications make the compound significantly more hydrophilic and thereby enhance excretion in the bile and urine. Phase III is often referred to as a "metabolic" process; however, it essentially involves the transport of xenobiotics and their metabolites across biological membranes and no further metabolic alterations of the compound's structure. An optimized drug should reach its target tissues at adequate concentration without excessive accumulation in other tissues particularly tissues of toxicological relevance for optimal drug efficacy and minimal drug toxicity. *The Intracellular concentration of successful drugs in the market is determined by the balance in the activity of multiple uptakes and efflux transporters that facilitate the drugs' movement across biological membranes.* Phase III transporters are found in a wide array of organs/biological membranes and they typically facilitate the removal of xenobiotics and their metabolites from the body. There are many different Phase III transporters with each one having specificity for different types of molecules (e.g. anions vs. cations). *In particular, drug transporters can be classified under two major super-families: solute carrier (SLC) and the ATP-binding cassette (ABC).* Transporters of the SLC superfamily are organic cation transporters (OCTs/SLC22A), the multidrug and toxin extrusion transporters (MATE transporters/SLC47A), the organic anion transporters (OATs/SLC22A), and the organic anion transporting polypeptides (OATPs/SLCO). Members of ABC

superfamily are important in drug toxicity, and these include P-glycoprotein (MDR1/ABCB1), multidrug resistance-associated protein (MRP/ABCC), and breast cancer resistance protein (BCRP/ABCG2). Transporters are important determinants for not just drug disposition and drug-drug interactions, but also becoming increasingly important influencing factors of efficacy and safety outcomes of a candidate drug. Examples include cisplatin affinity for OCT1 and OCT2 for transport is linked to its nephrotoxicity and ototoxicity. Statin transporter OATP2B1 present in muscle tissue plays a potential role for statin entry into muscle tissue and associated with the mechanism of statin-associated muscle toxicity.

Many Phase I and Phase II enzymes show significant interindividual variability, which leads to various levels of exposure to the reactive metabolites. There are two primary causes of this variation: polymorphism and environmental exposure. *Polymorphisms in drug metabolizing enzymes may cause dramatic differences in drug detoxification.* Several recent genome-wide association studies (GWAS) have been conducted based on the hypothesis that polymorphisms might play a role in determining the risk of Drug-Induced Liver Injury (DILI). The following therapeutic drugs were withdrawn from the market primarily because of hepatotoxicity: Troglitazone, Bromfenac, Trovafloxacin, Ebrotidine, Nimesulide, Nefazodone, Ximelagatran, and Pemoline.

Differences in expression, affinity, and activity in Phase I, Phase II enzymes and phase III transporters are noted in preclinical species used for efficacy and safety making extrapolation of *in-vitro* and *in-vivo* studies in preclinical species to that in humans. (Chu X et al., 2013, Wang L et al., 2015)

4. Forms of Hepatotoxin-Induced Liver Injury

Direct liver toxicity is generally identified readily in preclinical studies and/or the early clinical development and generally found to be acceptable when reversible and that the benefit far outweighs the potential risk as in cancer chemotherapy drugs and other lifesaving drugs. *Whereas the pathophysiology of idiosyncratic liver toxicity is multifactorial, involving drug/pharmacological factors, host factors, and factors affecting the adaptive immune system which may not be easily identified in preclinical studies.*

5. Mechanisms of Direct Hepatotoxicity

Hepatotoxins are taken up by hepatocytes and can injure the cell by damaging any of a number of cellular constituents. Typically, the parent compound is harmless until it is metabolized into a toxic intermediate. For example, acetaminophen is metabolized by the cytochrome P450 enzyme isoform2E1 to the toxic N-acetyl-p-benzoquinone amine. This potent oxidant is normally neutralized by glucuronide and sulfate conjugation. With large doses of acetaminophen, these protective factors become depleted and oxidative damage ensues. The creation of such oxidative stress is the final common pathway of cellular injury for a number of hepatotoxins in addition to acetaminophen. Metabolized hepatotoxins may act as oxidants themselves or cause the generation of reactive oxygen intermediates, such as superoxide and hydroxyl free radicals. These highly reactive compounds oxidize and alter cellular lipids,

proteins, or DNA, leading to cell injury, and death. The oxidative destruction of these cellular components has classically been thought to result in a form of cell death, termed necrosis. In necrotic death, the cell swells, the membranes rupture, and the cell disintegrate. However, oxygen radicals also induce hepatocyte death by apoptosis - an active process in which gene expression triggers a cell death marked by cell shrinkage and chromatin compaction. In both apoptosis and necrosis, death is regulated by cellular signaling pathways that are activated by toxin-induced changes in the redox homeostasis of the cell. Particularly important is activation of the mitogen-activated protein kinase pathway and the resultant activation of transcription factors, such as activating protein-1 and nuclear factor kB (NF-kB). These transcription factors presumably regulate the expression of genes that in turn promote or block cell death, although the identity of these genes remains unknown.

Numerous drugs cause DILI by inhibition of biliary excretion of conjugated bile salts mediated by BSEP, leading to several cases of drug withdrawals from the market and black-box warnings (e.g., troglitazone, bosentan, erythromycin, nefazodone).

6. Mechanisms of Idiosyncratic Hepatotoxicity

Idiosyncratic hepatotoxicity is likely to arise from complex interactions among genetic, nongenetic host susceptibility, and environmental factors. Nongenetic risk factors include age, sex, and other underlying diseases (eg, chronic liver disease or infection or inflammation). Compound-specific risk factors include daily dose, physicochemical properties, metabolism characteristics, and drug-drug interaction potential.

By definition, idiosyncratic reactions cannot be predicted from the outcomes of preclinical toxicity studies which may be due to significant differences in metabolism between the animal species tested and man, disease-related or genetic predispositions that are not present in animal studies. There may indeed be an immune-mediated idiosyncratic response in humans which is manifested by fever, rash, eosinophilia and multi-organ failure/involvement.

Some of the predisposing factors associated with idiosyncratic liver injury in humans (ALDEN ET AL., 2003) are

1. Structural alerts
2. Glutathione depletion
3. Glutathione drug/metabolite conjugates
4. Covalent binding
5. Bioaccumulation in the liver
6. Drug interactions
7. Toxicity gene induction responses
8. Ames or micronucleus assay positive response
9. Liver injury in the rat and/or higher species without a safety margin at steady-state concentration of drug in the liver
10. P450 enzyme induction and liver hypertrophy without a safety margin

7. Response of the Liver to Hepatocellular Injury

Following the destruction of the hepatic parenchyma, regeneration of parenchyma, fibrosis, and ductular proliferation (bile duct hyperplasia) may occur. *When hepatocyte destruction is limited and the reticulin network remains intact, regeneration with almost complete restitution of the liver structure can occur*. Severe parenchymal destruction with extensive loss of hepatocytes often is followed by ductular proliferation. With persistent parenchymal damage or extensive loss of hepatocytes and damage to the normal collagenous structure, fibrosis and post-necrotic scarring may occur which may result in regenerative parenchymal nodules. In areas of collapse and/or fibrosis, intrahepatic portovenous vascular shunts may form. Morphologic diagnosis should emphasize the primary or most important process (necrosis vs. inflammation) with appropriate modifiers indicating chronicity, severity, distribution, presence of inflammatory cells, and evidence of organisms (inclusion bodies, bacteria, fungi, etc). In some cases (ischemia, toxins, and certain viruses) zonal necrosis may be all that is seen in very acute conditions, but inflammation is likely to follow if the animal survives the initial insult. Similarly, in acute infections with some viruses and bacteria, there is very little inflammation in the very acute phase, but traditionally these conditions have been referred to as hepatitis. *Toxic liver injury manifestations of liver toxicity, one or more of which may occur with each toxin, include no morphologic abnormalities, hepatocellular swelling, lipidosis, necrosis (usually in a specific pattern), inflammation, and eventually fibrosis.* The pattern of necrosis depends on many factors and the identity of the toxin cannot be determined based on morphologic grounds alone and should be verified by toxicological testing. Patterned necrosis of the liver can also be caused by vascular disease, hypoxia, and some viruses and must be distinguished from necrosis due to toxins. Many hepatotoxins affecting are therapeutic agents that have idiopathic or idiosyncratic effects on the liver. Examples of acute toxicity include centrilobular to panlobular necrosis associated with benzodiazepines and those associated with trimethoprim sulfonamide and acute necrosis and inflammation associated with xylitol, carprofen, and amiodarone. Chronic intoxication, sometimes leading to cirrhosis has been associated with primidone, phenytoin, phenobarbital, and CCNU (Lomustine).

8. Inflammation

Hepatitis is inflammation of the liver parenchyma, whereas cholangitis is inflammation of the bile ducts. Inflammation is a complicated process involving the reaction to injury by blood vessels and the subsequent accumulation of fluid and leukocytes. Inflammation of the liver parenchyma can be a primary event or can follow injury to the hepatocytes. Injuries to the hepatic parenchyma may lead to reversible or irreversible injury. Reversible injury (cell swelling and lipidosis) rarely leads to inflammation; however, irreversible injury (cell death) is often followed by inflammation. Common types of cell death in the liver are apoptosis, coagulation necrosis, and liquefactive (lytic) necrosis. The outcome of a given hepatic insult depends on the nature, extent, and duration of the insult, and of course, survival of the host.

Hepatitis should be defined histologically by: severity, distribution, types of

inflammatory cells present (neutrophils, eosinophils, macrophages, lymphocytes, plasma cells), presence or absence of necrosis and apoptosis, type of necrosis (liquefactive or coagulative), pattern of necrosis (multifocal random, zonal, massive), regenerative response of hepatocytes and bile ducts, presence or absence of fibrin or thrombi, evidence of causative agents (inclusion bodies, bacteria, fungi, copper, etc.), and presence or absence of fibrosis. The pattern of injury and the response to injury may provide clues as to the cause and/or pathogenesis. The chronicity of inflammatory lesions may be difficult to determine as the line of demarcation between acute and chronic inflammation may be blurred.

9. Role of Kupffer Cells and Cytokines in Toxin-Induced Liver injury

One of the unique aspects of the cellular environment of the liver is that it contains 80 - 90% of the fixed macrophages in the body. During toxin-induced liver injury, additional macrophages are recruited from the circulation into the liver and these cells, along with the resident macrophages or Kupffer cells, undergo a morphological and physiological change, termed activation. Activated cells produce a number of products, including G reactive oxygen intermediates, proteolytic enzymes, nitric oxide, eicosanoids, and cytokines. Cytokines are a large family of small secreted proteins that exert biological effects on other cells. *Activated macrophages promote liver damage from hepatotoxins because the toxin-induced liver injury is markedly decreased when liver macrophages are depleted or functionally inactivated*. The actions of gut-derived lipopolysaccharide (LPS) are critical in this process becauseLPS neutralization also prevents much of the liver injury induced by toxins. LPS presumably promotes injury through its ability to activate macrophages. Although all of the previously mentioned products of activated macrophages may promote hepatocyte injury, experimental studies suggest that toxin-induced liver injury depends in large part on the effects of one cytokine, tumor necrosis factor a (TNFα). In animal models of liver injury, blocking the activity of TNFα dramatically reduces the amount of liver injury from a number of toxins, including alcohol and acetaminophen. Taken together, these findings suggest that during hepatotoxic injury LPS causes macrophage activation, leading to the production of TNFα, which then causes liver cell injury. Surprisingly, hepatotoxic liver injury in large part results therefore not from the direct effects of the toxin, but from the actions of TNFα produced by macrophages. The mechanism by which TNFα causes hepatocyte injury remains unclear. TNFα is normally toxic to tumor cells but not to nontransformed cells, including hepatocytes. Inhibition of hepatocyte RNA or protein synthesis sensitizes hepatocytes to injury and death from TNFα. Therefore, hepatocyte resistance to the toxic effects of TNFα depends on the induction by TNFα of a protective gene(s). Since hepatotoxins almost invariably interfere with the macromolecular synthesis, they presumably block expression of the protective gene, thereby sensitizing hepatocytes to death from TNFα. Although the identity of this protective gene in hepatocytes is currently unknown, it has been established that a critical factor in hepatocyte resistance to TNFα toxicity is the activation of the transcription factor NF-kB.

10. Biomarkers of Liver Toxicity

According to the recent version of FDA guidance for industry drug-induced liver injury: Premarketing clinical evaluation, it is recommended to use a combination of four tests as DILI biomarkers. Commonly measured serum biomarkers used in preclinical and clinical screening for hepatotoxicity are as follows.

a. Alanine Aminotransferase

Clinical chemistry data are routinely used for non-invasive monitoring of liver disease in preclinical species and humans, and alanine aminotransferase (ALT) is the most widely used clinical biomarker.ALT is responsible for the metabolism (transamination)of alanine and is found at much higher concentrations in the liver compared to other organs. *When the hepatocellular injury occurs, the liver-abundant enzyme ALT will leak into the extracellular space and enter the blood. An elevation of serum ALT activity is often reflective of liver cell damage.* Unfortunately, extrahepatic injury, such as muscle injury, can also lead to elevations in ALT, making ALT not entirely hepato-specific. Despite the fact that extrahepatic injury, such as muscle damage or cardiac injury, can lead to increases in ALT, serum ALT remains the most widely used and universally accepted biomarker for DILI. It is deemed to be the clinical chemistry gold standard for DILI detection and has long been used at the FDA to facilitate regulatory decision-making.

b. Aspartate Aminotransferase

Based on the same rationale as ALT, aspartate aminotransferase(AST) has also been introduced as a standard biomarker for DILI and is well accepted by clinicians. Similar to ALT, AST is responsible for the metabolism (transamination) of aspartate. *Even though the sensitivity of the AST test is believed to be lower than that of ALT, it is still a widely used liver biomarker.* Owing to its more ubiquitous expression in extrahepatic organs, such as the heart and muscle, AST is significantly less specific than ALT in detecting DILI.

c. Alkaline Phosphatase

Alkaline phosphatase (ALP) is an enzyme located in the liver, and its concentration in serum increases when the bile ducts are blocked. ALP is another diagnostic biomarker recommended in the FDA guidance and is widely adopted by clinicians. *More than the twofold isolated elevation of serum ALP, or an ALT/ALP ratio of no more than 2, as a key biomarker of cholestatic DILI.* It is noteworthy that conditions other than DILI, such as bone disease and pregnancy, are also associated with ALP elevation. Therefore, ALP should not be regarded as a specific biomarker of cholestatic DILI. The unique advantage of ALP is that it is at least partially predictive of biliary obstructive types of liver injury when used together with other DILI biomarkers.

d. Total Bilirubin

Total bilirubin (TBL) is a composite of unconjugated (extrahepatic) and conjugated (hepatic) bilirubin. Increased TBL causes jaundice and can indicate metabolism problems in the liver, for example, reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion. *Therefore, serum bilirubin concentration is a real liver function biomarker, which measures the ability of the liver to clear bilirubin from the blood as it circulates through the liver.* In contrast, serum transaminase levels indicate the rate of enzyme release from injured cells.

e. Other Hepatotoxicity Biomarkers

Some liver function tests are not sensitive or specific enough to be used as diagnostic biomarkers of hepatotoxicity but are elevated in severe liver diseases. These biomarkers are used primarily to confirm liver toxicity and indicate the extent of damage to liver function. Conventional biomarkers falling into this category are *gamma-glutamyl transferase (GGT), serum total protein(albumin), ammonia, cholesterol/triglycerides, fibrinogen, prothrombin time (prothrombin ratio and international normalized ratio), and urobilinogen.*

Elevated serum gamma-glutamyltransferase (GGT) activity can be found in liver disease and it has a similar profile as ALP in detecting disease of the biliary system. Generally speaking, ALP is the first test for the biliary disease and GGT provides a value to verify that the ALP elevations are due to biliary injury.

Following hepatic uptake of lipoprotein cholesterol (insoluble), a portion is enzymatically converted to bile salt (soluble). Only hepatocytes have cholesterol 7α-hydroxylase, which is the rate-limiting enzyme for the multiple process conversion. In acute hepatic necrosis, triglycerides may be elevated due to hepatic lipase deficiency. When the bile cannot be eliminated, cholesterol and triglycerides may accumulate in the blood as low-density lipoprotein cholesterol. *Because the liver is responsible for the production of blood coagulation factors, the clotting time will be increased due to the impaired synthesis in the liver.* However, it is not a sensitive biomarker because it only happens at the late stage of liver disease. Urobilinogen in urine is a colorless product of bilirubin reduction. In this respect, urobilinogen level has a similar role as bilirubin to indicate liver dysfunction. *Low urine urobilinogen may result from biliary obstruction or complete obstructive jaundice.* Because urobilinogen is formed in the intestine by bacteria, broad-spectrum antibiotic treatment can significantly decrease its level due to the damage of intestinal bacterial flora.

11. Preclinical Models for Hepatotoxicity Assessment

Current preclinical toxicity testing using rodents and nonrodents is effective to identify the most potent hepatotoxins. Quite often hepatotoxicity at extremely high doses in animals may not necessarily predict toxic potential in humans. *The outcome of several surveys indicates that there are compounds which show no significant evidence of liver toxicity in animals but cause liver toxicity in humans.* In such cases, it is particularly important to understand unique factors responsible for human differences from laboratory animal models and to modify models or testing strategies to account for such differences. Such factors include genetic variability, lifestyle factors, the formation of unique metabolites with hepatotoxic potential, immune-mediated events, drug-drug or drug-food or drug-environment interactions, or endogenous or exogenous factors that modify or compromise organ or tissue function. (FDA 2000).

Compounds that do not elicit significant hepatic injury in multiple animal species usually are not inducers of serious hepatotoxicity in man. Data from a study of adverse effects noted in preclinical models indicate that dogs rather than rats, mice, or rabbits are most predictive of similar outcomes in humans. As a rule, four weeks repeat dose testing that includes both primary and secondary pharmacology, as well as toxicity endpoints across at least2 species (one rodent and one nonrodent) is better predictive of a similar outcome in humans. *Immune-mediated hepatotoxicity can only rarely be predicted in preclinical studies*

Simultaneous use of multiple *in vitro* models including high content screening assays in cellular systems could improve results as a broader spectrum of molecular mechanisms would be encompassed, some of which might be associated with DILI. Although the specificity has been good with these models, the sensitivity was found to be medium when a larger set of drugs evaluated. The limited power of DILI prediction is mostly attributed to the complex nature of DILI, a poor understanding of mechanisms, a scarcity of human hepatotoxicity data.

A study involving 223 marketed drugs (51% associated with clinical hepatotoxicity; 49% non-hepatotoxic) to assess the concordance of *in vitro* bioactivation data with clinical hepatotoxicity (Sakatis MZ et al., 2012). Drugs with a daily dose of≥100 mg or with GSH adduct formation, marked P450 MDI, or covalent binding ≥200pmol eq/mg protein tended to be hepatotoxic (~ 65% in each case). Further, a combination of dose, solubility and lipophilicity criteria has been recommended to guide the selection of high-quality oral drug candidates for clinical development. The drugs classified as No DILI (n=163)had significantly lower dose and lipophilicity, and higher Fsp3 (fraction of carbon atoms that are sp3hybridised) versus the Most DILI (n=163) drugs. It is interesting to note that 59% of the most prescribed oral drugs are classified as Less DILI, suggesting that mild hepatotoxicity has not significantly restricted therapeutic use of effective drugs (Paul Leeson, 2018).

12. Conclusions

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity of these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market.

Hepatotoxicity and drug-induced liver injury also account for a substantial number of compound failures, highlighting the need for toxicity prediction models and drug screening assays that are capable of detecting toxicity early in the drug development process. Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests.

Hepatotoxins are synthetic or naturally occurring compounds that cause a variety of forms of liver injury through their direct or indirect damage to hepatocytes. Depending on the toxin, its likelihood to induce liver injury may be predictable or unpredictable (idiosyncratic). *The type of liver disease that may develop depends on the toxin and can range from only asymptomatic elevations in serum liver function tests to acute or chronic liver failure*. The mechanisms by which hepatotoxins injure the liver are complex and include both direct forms of biochemical cellular injury and indirect injury resulting from products of the accompanying inflammatory response.

Drug-induced liver injury (DILI) is the most common organ toxicity encountered

in regulatory animal toxicology studies required prior to the clinical development of new drug candidates. Very few reports have evaluated the value of these studies for predicting DILI in humans. Indeed, compounds inducing liver toxicity in regulatory toxicology studies are not always correlated with a risk of DILI in humans. *Conversely, compounds associated with the occurrence of DILI in phase 3 studies or aftermarket release are often tested negative in regulatory toxicology studies.* Idiosyncratic DILI is a rare event that is precipitated in an individual by the simultaneous occurrence of several critical factors. These factors may relate to the host (e.g. human leukocyte antigen polymorphism, inflammation), the drug (e.g. reactive metabolites) or the environment (e.g. diet/microbiota). This type of toxicity, therefore cannot be detected in conventional animal toxicology studies. Several animal models have recently been proposed for the identification of drugs with the potential to cause idiosyncratic DILI: rats treated with lipopolysaccharide, Sod2+/- mice, panels of inbred mouse strains or chimeric mice with humanized livers. These models are not suitable for use in the prospective screening of new drug candidates. Humans, therefore, constitute the best model for predicting and assessing idiopathic DILI.

13. Questions

1. *The functional group added by Phase I metabolism is often the target of _____conjugation.*
2. *The Intracellular concentration of successful drugs in the market is determined by the balance in the activity of _____ and _____ that facilitate the drugs' movement across biological membranes.*
3. *In particular, drug transporters can be classified under two major super-families: _____ and _____*
4. *Low urine urobilinogen may result from _____ or complete _____.*
5. *What are the biomarkers for Liver Injury?*
6. *_____measures the ability of the liver to clear bilirubin from the blood as it circulates through the liver*

14. References

Wong CH et al., Estimation of clinical trial success rates and related Parameters, Biostatistics. 2019 Apr 1;20(2):273-286

Waring MJ et al., An analysis of the attrition of drug candidates from four major pharmaceutical companies Nat Rev Drug Discov. 2015 Jul;14(7):475-86

Senior J, Drug hepatotoxicity from a regulatory perspective. Clin Liver Dis (2007) 11:507–524

Björnsson E, Hoofnagle J, Categorization of drugs implicated in causing liver injury: critical assessment based on published case reports. Hepatology (2016)63:590–603

Alden, C. et al. Predictive toxicology technology for avoiding idiosyncratic liver injury. Preclinica (2003). (May/June), 27–35.

Chu X et al., Species differences in drug transporters and implications for

translating preclinical findings to humans. Expert Opin Drug MetabToxicol. 2013 Mar;9(3):237-52.
FDA: Nonclinical Assessment of Potential Hepatotoxicity in Man,November 2000.
Paul D. Leeson,Impact of Physicochemical Properties on Dose and Hepatotoxicity of Oral Drugs Chem. Res. Toxicol.2018, 31,6, 494-505
Sakatis MZ et al., Preclinical strategy to reduce clinical hepatotoxicity using *in vitro* bioactivation data for >200 compounds. Chem Res Toxicol. 2012 Oct 15;25(10):2067-82.
Wang L et al., Interspecies variability in expression of hepatobiliary transporters across human, dog, monkey, and rat as determined by quantitative proteomics. Drug MetabDispos. 2015 Mar;43(3):367-74.

ToxGurukul Foundation

ToxGurukul Foundation is a registered non-profit organization for professionals in the field of toxicology who are in search of a platform to learn and share the vast knowledge in this area. This syndicate belongs to independent professionals from different backgrounds of toxicology who share their knowledge to un-puzzle the Rubik's cube that each face in their daily work routine.

Website: www.toxgurukul.org.in
Email: toxgurukul.india@gmail.com

Important Links – ToxGurukul Foundation

Follow us

Join us

Subscribe

Library

Feedback

ToxGurukul Foundation (A Non - Profit Organization)
Regd. Office: Fl. No.10, New Ajanta Avenue, Building-4, Wing- A1, S. No. 135/136 Part, Kothrud, Pune-411038
Corporate Identity Number: U80904PN2019NPL182886
Emai Id: toxgurukul.india@gmail.com

News/Updates

4th International Conference of the Asia QA Forum
September 5-6, 2019, Bengaluru.

GxP Regulations – Expectations, Challenges and Quality Focus for Global Needs

For more details : click here

3rd Interactive meet on Insights in Toxicology – 2019
Pathology for Toxicologists' - A ToxGurukul Initiative

(September 2019, Hyderabad)
More details will be announced soon.

To be a sponsor, please write to us
sponsor.toxgurukul@gmail.com

If you have any updates (conferences, job vacancies and so on.) to be shared here, please write to toxgurukul.india@gmail.com.