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DRUG INDUCED LIVER TOXICITY: A SURVEY

Mandeep Kaur, Dr. Shivanand. M. Patil
Research Scholar of Pharmacy Department, NIMS University, Jaipur.
K. T. Patil College of Pharmacy, Siddharth Nagar, Barshi Road, Osmanabad (MS).
Email: wellborneducation@gmail.com

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Abstract

Liver toxicity refers to damage done to the liver by medications and chemicals. The liver is an essential organ to the human body. Located on the right side of the body behind the ribs, the liver stores nutrients and produces proteins important to remain healthy. One of the main functions of the liver is to remove toxic substances from the bloodstream. This process may be interrupted if toxins begin to enter the bloodstream at a rate faster than the liver's ability to break them down, and this can cause liver toxicity. Many toxins target the liver and cause hepatotoxic effects that can be observed through some biochemical parameters. Impairment of the liver generally occurs from excessive exposure to xenobiotics, alcohol, chemotherapeutic agents, virus and protozoan infections. Depending upon the severity of toxicant insult, hepatic cell injury can lead from acute to chronic hepatitis, which if left untreated can result in cirrhosis or malignant lesions.

In this paper we survey the liver toxicity induced due to drug.

Keywords: liver toxicity, cytochrome, hepatotoxic, xenobiotics, alcohol, chemotherapeutic agents, virus, protozoan infections

1. Introduction

Drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease. The liver is the central organ for the metabolism of every foreign substance. Mostly all drugs are lipophilic. In hepatocytes drugs are rendered hydrophilic to yield water soluble products by biochemical processes which can be excreted in urine or bile. This process is called as biotransformation. It consists of phase 1 and phase 2 reactions. In the phase 1 reaction,

oxidation or demethylation occurs. These reactions are mediated by cytochrome P450. A variety of oxidative phase 1 reactions are performed by the enzymes that make up the P450 system. Typical phase 1 reaction will generate a hydroxyl group, which can then participate in phase 2 reactions. In phase 2 reactions, a large; water soluble polar group is introduced to hydroxyl oxygen by glucoronidation and or sulfation. Another metabolic pathway for detoxifying many compounds involves glutathione, a thiol-containing tripeptide, capable of binding to potentially harmful electrophilic compounds through glutathione *S*-transferase. This reaction is central to the detoxification of a number of compounds such as acetoaminophen (Lee., 2003)

1.1 Types of drug reactions

1.1. A) Direct Toxic Reactions

Acetaminophen is an example of an agent that causes a direct toxic reaction. It is used as a non narcotic pain reliever. The metabolic pathway for acetaminophen involves phase 1 and 2 reactions, glutathione detoxification, and the formation of reactive intermediates, which disrupt cellmacromolecules. Through CYP 450 an electrophilic compound, *N*-acetyl-p-benzoquinoneimine (NAPQI), is formed which can bind to covalently to cell macromolecules, thereby disrupting mitochondrial and possibly nuclear function. The formation of covalent bonds is prevented if NAPQI can be detoxified by conjugation (through glutathione-s-transferase) to generate, mercapturic acid, a harmless, water-soluble product excreted by the kidney. Depletion of glutathione reduces this last defense against the formation of NAPQI-related intracellular adducts. Thus, any situation that leads to the depletion of glutathione will increase toxicity, where as an increase in available glutathione stores will diminish this toxocity. Starvation and alcohol deplete mitochondrial glutathione, whereas *N*-acetylcysteine replenishes glutathione stores and protects against acetaminophen induced injury. Enzyme which is responsible for the conversion of acetaminophen to NAPQI, is induced by ethanol and inhibited by cimetidine.

Thus, at several metabolic stages, ethanol increases toxicity, whereas cimetidine may serve as an antidote.

1.1.B) Idiosyncratic Reactions

The majority of drug-related reactions, such as those observed with isoniazid, are idiosyncratic and unpredictable. Isoniazid is used for prophylaxis against the tuberculosis. Several factors explain the relatively common toxic reactions observed. First, the simultaneous use of alcohol or rifampin may augment the toxicity of

isoniazid. Second, elderly persons may be more likely to have toxic reactions than younger persons. Third, genetic differences also play an important role, since persons who are capable of rapid acetylation of isoniazid have an increased likelihood of toxic reactions resulting from the formation of acetylhydrazine, which is then transformed by cytochrome P450 into a reactive metabolite. Some studies suggest that persons with slow acetylation are at greater risk for a toxic reaction through a separate pathway that leads to the formation of hydrazine, which itself may be toxic.

1.1.C) Combined Toxic and Allergic Reactions

A halothane is a seldom-used anesthetic agent that can induce a combination of toxic and allergic reactions leading to liver injury. Severe halothane-related hepatitis generally develops after multiple exposures to the drug such as those that can occur on subspecialty surgical services. Protein adducts formed from the initial toxic reaction provide the hapten for the formation of antibodies, so that with subsequent exposure, antibody and cellular recognition of the halothane– protein-adduct antigen on the hepatocyte surface leads to cell lysis.

1.1.D) Allergic Hepatitis

Drugs such as phenytoin can cause a systemic allergic reaction characterized by fever, rash, lymphadenopathy,eosinophilia, and the presence of eosinophils or granulomas in liver-biopsy specimens. This allergic reaction is accompanied by both hepatocyte necrosis and cholestasis. The mechanisms responsible for the combined allergic and hepatotoxic reactions are not completely clear.

1.1.E) Cholestatic Reactions

The drugs that mainly affect bile flow, causing cholestatic injury, include estradiol, chlorpromazine, trimethoprim–sulfamethoxazole, rifampin, erythromycin estolate, nafcillin,and captopril .The mechanism of cholestatic injury remains unclear. Estradiol and other estrogens have been shown to decrease bile flow and Na+/K+-ATPase, change tight junctions between cells, and alter the fluidity of the hepatocyte membrane.

1.1.F) Drug-Induced Chronic Hepatitis

Methyldopa and a number of other compounds have been found to cause liver damage that closely resembles autoimmune chronic active hepatitis. The classic agent producing this reaction is oxyphenisatin, a laxative that has been withdrawn from the market. Early identification of such drug-related chronic hepatitis is not easy;

cirrhosis may develop before the hepatitis is diagnosed. Apart from this, identifying the drug or toxin that has caused the cirrhosis is difficult retrospectively if the patient has been consuming alcohol or if unrecognized viral hepatitis is present.

1.2 Mechanism of cellular injury

Injury to liver cells occurs in various patterns specific to the intracellular organelles affected. The normal hepatocytes can be injured in various manners such as

- High-energy reactions involving cytochrome P-450 enzymes lead to covalent binding of drug to intracellular proteins, producing intracellular dysfunction resulting in the loss of ionic gradients, a decline in ATP levels, and actin disruption, cell swelling, and cell rupture.
- Drugs that affect transport proteins at the canalicular membrane can interrupt bile flow.
- Certain drugs bind to or disable the bile salt export protein. This process causes cholestasis.
- Disruption of intracellular calcium homeostasis leads to the disassembly of actin fibrils at the surface of the hepatocyte, resulting in blebbing of the cell membrane, rupture, and cell lysis.
- Drugs are relatively small molecules and, therefore, rarely evoke an immune response.

However, biotransformation involving high-energy reactions can result in the formation of adducts that is, drugs covalently bound to enzymes. Adducts that are large enough to serve as immune targets may migrate to the surface of the hepatocyte, where they can induce the formation of antibodies (antibody mediated cytotoxicity) or induce direct cytolytic T-cell responses .The secondary cytokine response thus evoked may cause inflammation and additional neutrophil-mediated hepatotoxicity.

- Programmed cell death (apoptosis) can occur along with immune-mediated injury. This can destroy hepatocytes through tumor necrosis factor (TNF) and the Fas pathways, with cell shrinkage and fragmentation of nuclear chromatin. Proapoptotic receptor enzymes, if activated by drugs, will compete with survival pathways within the cell, and this dynamic interaction may shift the balance either in favor of or against further cell damage.
- Still other pathways to injury may develop when drugs damage to mitochondria, disrupting fatty-

acid oxidation and energy production. When drugs bind to respiratory- chain enzymes or mitochondrial DNA, oxidative stress results, with ensuing anaerobic metabolism, lactic acidosis, and triglyceride accumulation

- Other cells within the liver may be the target of drug injury or serve as modulators of other reactions. For example, Kupffer's cells activate cytokines that may amplify injury, and fat-storage cells (stellate cells) or macrophages may augment injury, produce fibrosis, or form granulomas.

2. Selected Liver Toxicants & Drugs

2.2.1 Carbon tetra chloride (CCl_4)

Liver injuries induced by CCl_4 are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and/ or hepatoprotective activities of drugs [2]. It has been established that CCl_4 is accumulated in hepatic cells and metabolically activated by cytochrome P450 dependent monooxygenases to form a trichloromethyl radical (CCl_3). This radical can either covalently bind to a variety of hepatic macromolecules or initiate lipid peroxidation. Either of these processes could lead to the denaturation of critical metabolic pathways with ensuing toxicity. This model is supported by a variety of studies, including investigations demonstrating *in vivo* covalent binding of radiolabeled CCl_4, to cellular macromolecules, alterations in the toxicity of CCl_4 and manipulations that are known to affect the activity of the cytochromes P-450 pathways. Further reconstitution studies with purified cytochromes P-450 have confirmed their role in the reduction of CCl_4.

Juhad et al.,[3] were the first to suggest that cell death from CCl_4, was due to alterations in Ca^{2+} metabolism. Increase in cytosolic free [Ca^{2+}] leads to the activation of a variety of hydrolytic enzymes and ultimately leads to cell death. Further evidence has indicated that the cytochrome P-450 system and Ca^{2+} pump are in close proximity to each other on the microsomal membrane. This proximity could facilitate the toxic process. These studies suggest that damage to the ATP-dependent microsomal Ca^{2+} pump may be the critical event in initiating CCl_4 toxicity. Since the above mechanisms will have a direct or indirect effect on mitochondria, we

have extended our investigations to study the expression of genes related to mitochondrial changes and their molecular mechanisms before and after CCl_4 intoxication.

2.2 D-Galactosamine (D GalN)

D-galactosamine is a well established hepatotoxicant induces a diffuse type of liver injury closely resembling human viral hepatitis. A single injection with d- galactosamine can decrease the uracil nucleotides in the liver and heart [4]. Galactosamine markedly depletes hepatic UDP glucuronic acid (UDP-GA) whereas extrahepatic UDP-GA is minimally affected. This suggests that GAL predominantly inhibits hepatic glucuronidation. It disrupts the synthesis of essential uridylate nucleotides resulting in organelle injury. Depletion of these nucleotides ultimately impairs the synthesis of protein and glycoprotein, leads to progressive damage of cellular membranes resulting in a change in permeability of the cellular membrane which leads to enzyme leakage from the cells [5].

Although D-GalN has been known as a hepatotoxin causing necrosis, it has also been reported to induce apoptosis in the liver of rats [6]. As it is known, DNA fragmentation is an indicator of apoptotic cell death . The high incidence of apoptosis in D-GalN intoxication was explained on the basis that toxicity of D-GalN is mediated through tumor necrosis factor (TNF α, which causes apoptosis in liver cell [7]. TNF α is synthesized in the Kupffer cells and may be responsible for induction of apoptotic and necrotic cell death of hepatocytes[8]. While there is a lot of information on intracellular effects induced by TNF α, its mechanism of cytotoxicity is still unknown. Several studies indicate that oxygen radicals may mediate some of the effects of TNF α taken together these observations, we included in our study whether D-GalN could induce oxidative stress and necrosis/or apoptosis via intrinsic pathway i.e. (involving mitochondrial damage).

2.3 Alcohol

Alcohol is a direct systemic toxin although it is widely consumed in the world. Chronic alcohol consumption leads to several metabolic disorders including hepatic and extra hepatic diseases [9]. The first step in the metabolism of alcohol is the oxidation of alcohol to acetaldehyde catalyzed by alcohol/dehydrogenase containing the coenzyme NAD^+. The acetaldehyde is further oxidized to acetic acid and finally CO_2 and water through the citric acid

cycle. A number of metabolic effects from alcohol are directly linked to the production of an excess of both NADH and acetaldehyde.

A central role in the toxicity of alcohol may be played by acetaldehyde itself. Although the liver converts acetaldehyde into acetic acid, it reaches a saturation point where some of it escapes into the blood stream. The accumulated acetaldehyde exerts its toxic effects by inhibiting the mitochondria reactions and functions. A high acetaldehyde level impairs mitochondria function; metabolism of acetaldehyde to acetic acid decreases, more acetaldehyde accumulates, and causes further liver damage.

Various studies have proved that alcohol is metabolized in the liver mainly by cytochrome P450 2E1 into various reactive oxygen species (ROS), e.g. 1-hydroxyethyl radical, hydroxyl radical, and superoxide radical [10]. These harmful species are known to cause oxidative degeneration of cellular molecules, which results in cell injury and dysfunction in liver and other organs. [11]

Investigations have suggested that alcohol causes direct damage to the liver mitochondrial membrane [12]. Hence in our studies we have determined the effect of alcohol on the expression of genes related to mitochondrial damage and induction of cell death.

2.4 Paracetamol (Acetaminophen)

Paracetamol is widely used as an analgesic and antipyretic agent, but it can produce severe hepatic injury when an overdose occurs. In therapeutic doses, about 80% of paracetamol is conjugated directly and forms sulfate and glucuronide esters before oxidation, and these conjugated esters are excreted in bile or urine. However a small amount of paracetamol is converted by hepatic cytochrome P450 (CYP2E1) to a highly reactive and toxic quinone intermediate, N-acetyl-para-benzo-quinoneimine (NAPQI). This intermediate (NAPQI) is known to bind covalently to intracellular macromolecules, deplete glutathione, cause oxidative stress, and alter calcium and/or thiol status in liver cells, all leading to hepatocellular injury. In toxic doses, paracetamol causes acute centrilobular hepatic necrosis with collapse of the reticulin.

It is generally accepted that the ultimate form of hepatic damage caused by paracetamol is necrosis [13]. However, several reports have presented evidence for the occurrence of apoptosis in paracetamol induced hepatic damage. For instance, Ferret and co-workers have shown that caspase-3 and 9 activities were slightly increased in

mice that were administered an hepatotoxic dose of paracetamol, possibility of direct induction of apoptosis by the cytotoxic metabolite of paracetamol. *N*-acetyl-*p*-benzoquinone imine, additional factors, cytokines such as tumor necrosis factor and CD95 ligand have been implicated in paracetamol induced liver damage [14]. Considering the above facts, paracetamol was selected to screen its effect on mitochondrial damage leading to apoptosis or necrosis.

2.5 Isoniazid INH), Rifampicin (RIF) and Pyrazinamide (PYZ)

Anti tubercular (AT) drugs are the commonest agents causing serious, clinically significant drug induced liver disease in the developing countries. Most commonly used AT drugs like Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PYZ) are hepatotoxic. Various factors predisposing to ATD hepatotoxicity, both genetic and acquired, are well delineated [15]. The hepatotoxicity of INH is thought to be initiated by cytochrome P450 (CYP) mediated metabolism of INH to acetylhydrazine and hydrazine. RIF, which is generally co-administered with INH in the treatment of tuberculosis, enhances hydrazine by enzyme induction. The high reactivity of hydrazine with sulfhydryl groups results in glutathione depletion within the hepatocytes leading to cell death.

A few experimental studies have also demonstrated the critical role of glutathione in AT drugs induced hepatotoxicity. But only depletion of hepatic glutathione by INH or its metabolites, could not explain the AT drug induced hepatotoxicity.

Mitochondrion is an important organelle for cell survival and functions. Mitochondrial dysfunctions have been observed in diclofenac hepatotoxicity. Mitochondrial permeability transition (MPT) is focused as a mechanism for drug induced liver cell injury [16], causing both necrotic and apoptotic cell death. INH could produce apoptosis in Hep G2 and AHH1 cell line [17], as well as necrosis in rabbit liver. However, there is lack of experimental data that supports the role of mitochondria in AT drugs induced liver injury. In the present study, we have investigated the possible role of mitochondria, particularly oxidative stress and involvement of MPT in AT drugs induced liver cell injury.

3. Selected Hepatoprotective Agents

There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity[18]. Nearly 150

phytoconstituents from 101 plants have been claimed to possess liver protecting activity, but only some of them are still in traditional use. The following hepatoprotective drugs are considered as standards which are already available in the pharmaceutical market. Since, our main objective is focused on the gene expression profile of Bax, Bcl2 and their role on mitochondrial damage, it is very important to study the effect of different hepatoprotective drugs before and after toxicant treatment. Hence we have selected the following hepatoprotective drugs to be included in the objective.

3.1 Silymarin

Silymarin isolated from the seeds of *Silybum marianum* (Asteraceae) is a mixture of flavanolignans - silybin, silidianin and silychristin **.** The extract also contains other flavonoids, mainly taxifolin and quercetin [19] . All these compounds account for 65–80% of the whole extract content, with the remaining fraction being a chemically not well-defined fraction, composed mostly of polymeric and oxidized polyphenolic compounds . The mechanisms by which silymarin exerts its hepatoprotective action are under intensive investigation, and appear to be multifactorial. Silymarin and silybin prevent lipid-peroxide formation in liver cells, mainly due to their free- radical-scavenger properties. Silymarin also has antifibrinogenic properties, and is able to increase the synthesis rate of rRNA by activating RNA polymerase I; this enhances the biosynthetic apparatus, thus increasing the synthesis rate of both structural and functional proteins. Other than its anti oxidant action, silymarin also reduces the turnover of membrane phospholipids and stabilizes the cell membranes of hepatocytes. Silymarin is prescribed under category as liver protectant and used in the treatment of hepatic disorders. Few marked brands of silymarin are Silybon (Micro labs), Silimar (Zy.Cad) and Sivylar (RanBaxy).

3.2 Lecithin

Lecithin, an important phospholipid is found in the major organs in our body such as the heart, the liver, and the kidneys. Lecithin, a component of most cells, will help in transport and responsible for overall health of the body. Though it is produced within our own bodies, we do not always consume enough of the nutrition needed to produce it in adequate amounts.

Lecithin is composed of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides and phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. However, lecithin is sometimes

used as a synonym for pure phosphatidylcholine a phospholipid that is the major component of its phosphatide fraction. It may be isolated either from egg yolk or soya beans. Role of lecithin as hepatoprotective drug have been well studied and reported [20][21]. Lecithin is used in combination with silymarin (1:1 molar ratio) marketed as silipide. The efficacy of silipide in cases of chronic hepatitis patients was found superior to silymarin (silybin) due to higher bioavailability.

3.3 Catechin

Fresh tea leaves are rich in flavanol monomers known as catechins . Flavonoids have been found to play a very important role in protection against oxidative stress [22]. Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables, tea, cocoas and red wine [23]. Studies have been carried out exploring different biological activities of catechins. Scavenging and productive activities of green tea polyphenols [24], preventive effect of green tea catechins on experimental tumor metastasis [25], protective effect of green tea on ethanol induced lipid peroxidation and tamoxifen-induced liver injury [26] have been reported. There have been reports supporting the hepatoprotective effects of green tea against ethanol intoxication [27]. Effect of green tea and epigallocatechin gallate on ethanol-induced toxicity in HepG2 Cells was also reported [28]. Proposed mechanism of catechin is its ability as powerful antioxidant and its free radical scavenging property.

3.4 L-Ornithin and L-Aspartate (LOLA)

L-Aspartate is an amino acid found in all forms of life. L-Aspartate is a dicarboxyl amino acid found in small amounts in body fluids. The body can synthesize l-aspartate, which makes it a non essential amino acid. L-Aspartate serves as a precursor for synthesis of proteins, oligopeptides, purines, pyrimidines, nucleic acids and L-arginine. L-aspartate is important for the proper functioning of the body. Supplementation may be necessary if the body, due to physical strains or conditions, is unable to produce the required amount.

Ornithine, an amino acid, is manufactured by the body when another amino acid, arginine, is metabolized during the production of urea. Since ornithine is produced by the body, a deficiency of this nonessential aminoacid can occur only during adverse conditions like severe trauma or malnutrition or some time during pregnancy.

L Ornithin and L aspartate is available with trade name such as Hepacor (Intas), Lornit infusion (Zuventus) and Hepalon (Micro Nova). Ornithine aspartate has been shown to be beneficial in people with hepatic encephalopathy

and liver cirrhosis. In a double-blind trial, people with cirrhosis and hepatic encephalopathy received either L-ornithine-L-aspartate or a placebo for two weeks. Those taking the ornithine had significant improvements in liver function and blood tests compared with those taking the placebo.

It is widely believed that ammonia plays an impotant role in the multifactorial pathogenesis of hepatic encephalopathy. For all these reasons, reducing hyperammonia in patients with severe liver failure has always been one of the important goals of therapeutic applications. Administration of omithine and also ornithine compounds has been proved to decrease blood ammonia concentrations. During the last decades, clinical studies have shown that L-omithine- L-aspartate reduces blood ammonia concentration, restores amino acid imbalances and may improve the clinical symptoms of hepatic encephalopathy in patients with mild liver failure.

4. Conclusion

Drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease the liver is the central organ for the metabolism of every foreign substance. Mostly all drugs are lipophilic. In this paper we reviewed the drug related reactions.

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Corresponding Author:
Dr. Shivanand. M. Patil
Email: *wellborneducation@gmail.com*