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Experimental models used for the study of antihepatotoxic agents

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ABSTRACT

Both *in vitro* and *in vivo* liver models have been developed in the past years to study the hepatoprotective agents. These systems measure the ability of the test drug to prevent or cure liver toxicity (induced by various hepatotoxins) in experimental animals. In *in vitro* models fresh hepatocytes are treated with hepatotoxin and the effect of the test drug on the same is evaluated. In *in vivo* models, a toxic dose or repeated doses of a known hepatotoxin are administered to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. Various chemical agents normally used to induce hepatotoxicty in experimental animals for the evaluation of hepatoprotective agents include carbon tetrachloride, paracetamol, Acrylamide, adriamycin, alcohol, antitubercular drugs *etc*. The present article explains the mechanism of action of various hepatotoxic chemical/drugs, their dosage and route of administration.

1. Introduction

The liver is a vital organ present in vertebrates and some other animals, and is typically the largest visceral organ. The liver plays a major role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Some medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins[1]. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug−induced hepatotoxicity represents a major clinical problem accounting for 50% of all cases of acute liver failure. Although the majority of cases of acute liver failure are due to intentional or unintentional misuse, 16% are idiosyncratic[2]. Some of the inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally− occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. In addition, exposure to hepatotoxic compounds may be occupational,

environmental or domestic that could be accidental, homicidal or suicidal ingestion[3].

2. Hepatotoxicity

Drugs continue to be pulled from the market with disturbing regularity because of late discovery of hepatotoxicity[4]. The mechanism of hepatic injury has been proposed to involve 2 pathways-direct hepatotoxicity and adverse immune reactions. In most instances, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress. Additionally, these reactive metabolites may induce disruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production. Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells[5]. Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses. Stress and damage to hepatocytes result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC), natural killer

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(NK) cells, and NKT cells. These cells contribute to the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor (TNF)–α, interferon (IFN)–γ, and interleukin (IL)–1β, produced during hepatic injury are involved in promoting tissue damage[6]. However, innate immune cells are also the main source of IL–10, IL–6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role[7]. It is, therefore the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the innate immune system that determines an individual's susceptibility and adaptation to hepatic injury.

3. Evauluation of antihepatotoxic agents

The therapeutic value, efficacy and toxicity of drugs may be evaluated in animals experimentally made sick, followed by clinical trials. Detailed biochemical and other *in vitro* assays are obligatory to establish the mechanism of action. Both *in vivo* and *in vitro* test systems are employed to assess antihepatotoxic or hepatoprotective activity. These systems measure the ability of the test drug to prevent or cure liver toxicity (induced by various hepatotoxins) in experimental animals (rats, mice *etc*.) [8].

4. *In vitro* models

Fresh hepatocyte preparations and primary cultured hepatocytes are cultured to study the anti–hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the test drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An augmented activity of marker transaminases in the medium indicates liver damag. Parameters such as hepatocytes multiplication, morphology, macromolecular synthesis and oxygen consumption are determined[9].

5. *In vivo* models

A toxic dose or repeated doses of a known hepatotoxin is administered to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. Liver damage and recovery from damage are assessed by quantifying serum marker enzymes, bilirubin, bile flow, histopathological changes and biochemical changes in liver. An augmented level of liver marker enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase in the serum indicates liver damage[10]. Therapeutic efficacy of a drug against diverse hepatotoxins differs especially when their mechanism of action vary. Consequently, the efficacy of each drug has to be tested against hepatotoxins which act by varied methods.

6. Hepatotoxic agents

Various chemical agents normally used to induce hepatotoxicty in experimental animals for the evaluation of hepatoprotective agents are:

6.1. Acryl amide

Acrylamide (AA) is a water–soluble vinyl monomer used in the production and synthesis of polyacrylamides. Monomeric AA has been shown to cause diverse toxic effects in experimental animals. Acrylamide is carcinogenic to laboratory rodents and is described by the International Agency for Research of Cancer as a probable carcinogen to humans. In the human body, AA is oxidized to the epoxide glycidamide (2,3–epoxypro–pionamide) via an enzymatic reaction involving cytochrome P4502E1. AA undergoes biotransformation by conjugation with glutathione and is probably being the major route of detoxification. Daily dose of 6 mg/kg, ip for 15 d produces hepatotoxicity in female Sprague–Dawley rats[11].

6.2. Adriamycin

Adriamycin (doxorubicin) is an antibiotic isolated from streptomyces peucetius var Cesius Adriamycin and is considered to be one of the most compelling drugs against a wide range of tumors. However, its clinical potential is contraindicated due to severe cytotoxic side effects Based on *in vitro* model of toxicity using isolated hepatocytes and liver microsomes, adriamycin has been shown to undergo redox cycling between semiquinone and quinone radicals during its oxidative metabolism. A single dose of 10 mg/kg body weight of adriamycin is given to rats to induce hepatotoxicity[12].

6.3. Alcohol

Liver is among the organs most susceptible to the toxic effects of ethanol. Alcohol consumption is known to cause fatty infiltration, hepatitis and cirrhosis. Fat infiltration is a reversible phenomenon that occurs when alcohol replaces fatty acids in the mitochondria. Hepatitis and cirrhosis may occur because of enhanced lipid peroxidative reaction during the microsomal metabolism of ethanol. The mechanisms responsible for effects of alcohol, an increase in hepatic lipid peroxidation leads to alteration in membrane phospholipid composition. The effects of ethanol have been suggested to be a result of the enhanced generation of oxy free radicals during its oxidation in liver. The peroxidation of membrane lipids results in loss of membrane structure and integrity. These results in elevated levels of glutamyl transpeptidase, a membrane bound enzyme in serum. Ethanol inhibits glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in

levels of glutathione in liver[13]. The decrease in activity of antioxidant enzymes superoxide dismutase, glutathione peroxidase are speculated to be due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, formed by oxidation of ethanol. Continuous administration of ethanol (7.9 g/kg body weight/d) for a period of 6 weeks induces liver damage in rats[14].

6.4. Alpha–naphthylisothiocyanate

Alpha–Naphthylisothiocyanate (ANIT) injures bile duct epithelium and hepatic parenchymal cells in rats. It is commonly believed that ANIT undergoes bioactivation by hepatic, cytochrome P450–dependent mixed–function oxidases. Rats intoxicated once with ANIT (75 mg/kg, intraperitoneal (i.p.)) show liver cell damage and biliary cell damage with cholestasis at 24 h, but not 12 h, after intoxication[15].

6.5. Antitubercular drugs

Drug induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular therapeutic regimens containing Isoniazid (INH), Rifampicin and Pyrazinamide. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimens. Thus, though INH, Rifampicin and Pyrazinamide each in itself are potentially hepatotoxic, when given in combination, their toxic effect is enhanced. INH is metabolized to monoacetyl hydrazine, which is further metabolized to a toxic product by cytochrome P450 leading to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis[16]. This has been postulated due to rifampicin–induced cytochrome P450 enzyme–induction, causing an increased production of the toxic metabolites from acetyl hydrazine (AcHz). Rifampicin also increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination. Rifampicin induces hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine. Pharmacokinetic interactions exist between rifampicin and pyrazinamide in tuberculosis patients, when these drugs are administered concomitantly. Pyrazinamide decrease the blood level of rifampicin by decreasing its bioavailability and increasing its clearance. Pyrazinamide, in combination with INH and rifampicin, appears to be associated with an increased incidence of hepatotoxicity[17].

6.6. Cadmium

Cadmium, a heavy metal well known to be highly toxic to both humans and animals, is distributed widely in the environment due to its use in various industries. Some of the toxic effects of cadmium exposure are testicular atrophy, renal dysfunction, hepatic damage, hypertension, central nervous system injury and anemia. Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles[18]. Cadmium depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals and hydrogen peroxides. These reactive oxygen species result in increased lipid. Cadmium is given orally (3 mg/kg body weight/d) as cadmium chloride ($CdCl_2$) for 3 weeks to induce hepatotoxicity in rats[19].

6.7. Carbon tetrachloride

Carbontetrachloride is metabolized by cytochrome P–450 in endoplasmic reticulum and mitochondria with the formation of CCl_3O^- , a reactive oxidative free radical, which initiates lipid peroxidation.
Administration of a single dose of CCl_4 to a rat produces, within 24 h, a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 h of administration. Thereafter, the level falls and by 24 h there is no CCl_4 left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum. Dose of CCl_4: 1 mL/kg body weight, i.p., 1:1 v/v mixture of CCl_4 and olive oil[20].

6.8. Erythromycin

Erythromycin estolate is a potent macrolide antibiotic, generates free radicals and has been reported to induce liver toxicity. Erythromycin when given as erythromycin stearate (100 mg/kg body weight for 14 d)[21] or erythromycin esolate (800 mg/kg/d for 15 d) to albino rats produces hepatotoxicity in them[22].

6.9. Galactosamine

Galactosamine produces diffuse type of liver injury simulating viral hepatitis. It presumably disrupts the synthesis of essential uridylate nucleotides resulting in organelle injury and ultimately cell death. Depletion of those nucleotides would impede the normal synthesis of RNA and consequently would produce a decline in protein synthesis. This mechanism of toxicity brings about an increase in cell membrane permeability leading to enzyme leakage and eventually cell death. The cholestasis caused by galactosamine may be from its damaging effects on bile ducts or ductules or canalicular membrane of hepatocytes Galactosamine decrease the bile flow and its content i.e. bile salts, cholic acid and deoxycholic acid. Galactosamine reduces the number of viable hepatocytes as well as rate

of oxygen consumption. Hepatic injury is induced by intraperitoneal single dose injection of D–galactosamine (800 mg/kg) [23].

6.10. Lead

Lead is known to disrupt the biological systems by altering the molecular interactions, cell signaling, and cellular function. Exposure to even low levels of lead may have potential hazardous effects on brain, liver, kidneys and testes. Autopsy studies of lead–exposed humans indicate that among soft tissue, liver is the largest repository (33%) of lead, followed by kidney. Lead–induced hepatic damage is mostly rooted in lipid peroxidation (LPO) and disturbance of the prooxidant–antioxidant balance by generation of reactive oxygen species (ROS). Hepatotoxicity can be induced by using lead acetate (550 ppm for 21 d in drinking water)[24] or lead nitrate (5 mg/kg body weight daily for 30 d) [25].

6.11. Microcystin

Microcystin–LR, a cyclic heptapeptide synthesized by the blue–green algae, Microcystis aeruginosa, is a potent hepatotoxin. Pathological examination of livers from mice and rats that received microcystin–LR revealed severe, peracute, diffuse, centrilobular hepatocellular necrosis, and hemorrhage. Mice receiving sublethal doses of microcystin (20 μ g/kg) for 28 weeks developed neoplastic liver nodules[26].

6.12. Paracetamol

It is a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. The covalent binding of N–acetyl–P–benzoquinoneimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver. Dose of Paracetamol: 2 g/kg P.O[27].

6.13. Phalloidin

Phalloidin, is one of the main toxins of Amanita phalloides. It induces hepatotoxicity in rats at an intravenous dose of 50 μ g/100 g b.w. Phalloidin also induces a cytolytic lesion. Phalloidin causes severe liver damage characterized by marked cholestasis, which is due in part to irreversible polymerization of actin filaments. Liver uptake of this toxin through the transporter OATP1B1 is inhibited by the bile acid derivative BALU–1, which does not inhibit the sodium–dependent bile acid transporter NTCP[28].

6.14. Tamoxifen

Tamoxifen citrate (TAM) is a non–steroidal antiestrogen drug used in treatment and prevention of hormone dependent breast cancer. In high dose, it is a known liver carcinogen in rats, due to oxygen radical overproduction and lipid per oxidation via formation of lipid peroxy radicals. An ip dose of 45 mg/kg/d of tamoxifen citrate in 0.1 mL dimethylsulfoxide and normal saline for 6 d induce hepatotoxicity in rats[30].

6.15. Tert–Butyl hydroperoxide (t–BHP)

Hepatotoxicity and oxidative stress is induced in male rats at various times (0–24 h) after t–BHP (0, 0.2, 0.5, 1 or 3 mmol/kg, ip) treatment. Serum hepatotoxicity parameters have been reported to increase from 2 h following 1 mmol/kg t–BHP and maximum values at 8 h. The elevation of hepatotoxic parameters and plasma MDA has been observed from 0.5 to 1 mmol/kg t–BHP, respectively, in a dose–dependent manner. Being a short chain analog of lipid peroxide, t–BHP is metabolized into free radical intermediates by cytochrome P450 in hepatocytes, which initiate lipid peroxidation, glutathione depletion and cell damage[30].

6.16. Thioacetamide induced

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. A metabolite of thioacetamide (perhaps s–oxide) is responsible for hepatic injury. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content i.e. bile salts, cholic acid and deoxycholic acid. I.P. dose of thioacetamide: 200 mg/kg, thrice weekly for 8 weeks induces hepatotoxicity[31].

7. Conclusion

Drug discovery and development consists of a series of processes starting with the demonstration of pharmacological effects in experimental cell and animal models and ending with drug safety and efficacy studies in patients. A main limitation is often the unacceptable level of toxicity with the liver as the primary target organ. The study of hepatoprotective agents has thus become an important field of research. This article explains various types of hepatotoxins which are employed in the study the the antihepatotoxic or hepatoprotective agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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