Review Article

Different methods for the induction of hepatotoxicity in experimental animals: A brief review

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Abstract

The liver is the body's largest and most essential organ, involved in the metabolism to food and medications. Humans are at risk to dying from liver illnesses. During drug development, pre-clinical research, and clinical treatment, liver damage is a significant reason to drug approval withdrawal. Patients with significant liver injury may have abrupt liver failure or possibly death if not appropriately treated. A deeper knowledge to primary mechanisms is required for the development to new therapeutic medicines. As a result, animal models that mirror human liver illnesses are being produced. For decades, animal models have been utilised to research the pathophysiology to liver illnesses and related toxicity. Various animal models were revealed in this review. Our primary goal is to investigate all new and conventional animal models that are classified as non-invasive and generate hepatotoxicity.

Keywords: Liver, hepatotoxicity, animal model, therapeutic medicines, injury, non-invasive

Introduction

The liver, which serves as a centre for the metabolism to nutrients and the excretion to waste metabolites, is the biggest solid organ, the largest gland, and one to the most important organs (Anthea, 1993). Before being distributed to the systemic circulatory system, its primary function is to control the flow and security to substances received from the digestive system (Ostapowicz, 2002).

Hepatotoxicity is the term used to describe liver impairment caused by various medications and substances. Medicinal medicines are metabolized in the liver into chemically reactive compounds that can interact with cellular macromolecules such as protein, lipids, and nucleic acids, causing protein malfunction, lipid peroxidation (LPO), DNA damage, and oxidative stress. This cellular function damage can result in cell death and likely liver damage. Over 75% to atypical medication reactions result in liver transplantation or death (Ostapowicz, 2002). The liver is essential in the regulation to several physiological processes (Ahsan et al., 2009**).** The cytochrome p 450 system's CYP2E1 enzyme, which contributes to oxidative stress, was elevated by these hepatotoxic medications. Bile acid builds up inside the liver due to damage to hepatocytes and bile duct cells, which encourages more liver damage. The activation to the innate immune system, such as Kupffer cells (KC), natural killer (NK) cells, and NKT cells, produces pro-inflammatory mediators such as tumor necrosis factor-, interferon-, and interleukin-produced liver damage. Many chemicals and substances cause mitochondrial damage, which is an internal organelle that creates energy. Hepatocellular death in mitochondria directly results from medicines acting on these organelles. These programs cause necrosis or apoptosis and are mediated by signaling systems that arise at the cell membrane (for example, death receptors) (Patel et al., 1998). To discover a viable pharmaceutical target for hepatotoxicity, many experimental models are used to produce hepatotoxicity. However, there is currently little literature on animal models to hepatotoxicity. This review focuses on hepatotoxicity induced by various experimentally created animal models (Uetrecht, 2006).

Animal models are an important tool for studying mechanisms in almost all biomedical studies (Liu et al., 2013). They entail the intricacy to the entire animal, making in

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vivo system monitoring difficult. As the compounds are exposed in a sequential manner through absorption from the initial exposed location followed by metabolism, distribution, and elimination, an in vivo system fully reflects the exposing profile and cellular function. However, it must use essentially the same mechanism as human reactions, and the detrimental effect must be clinically significant. Small animals such as rats, mice, rabbits, and guinea pigs, as well as large animals such as pigs, cattle, sheep, and monkeys, are both useful and trustworthy for researching hepatotoxic effects, distribution, and clearance. They have the potential to be utilised to unravel the basic mechanism to xenobiotic actions, which will aid in understanding their influence on human health. The experimental model, on the other hand, serves as a road map for the discovery to novel molecular, noble signalling pathways for the benefit to the human race (Olson et al., 2000; Dambach et al., 2005).

In vitro hepatotoxicity methods are commonly used to assess the hepatotoxicity to drugs/chemicals/biological in order to understand the mechanism (s) and establish their relationship with in vivo hepatotoxicity. The current review focuses on the likely mechanism (s) to hepatotoxicity caused by various toxicants (paracetamol, carbon tetrachloride, alcohol, d-galactosamine, lipopolysaccharides, aflatoxin-B1, thioacetamide, cisplatin, arsenic, doxorubicin, cyclosporine-A), alterations in different cell compartments (Gomez-Lechon Maria et al., 2010).

There have been several studies on hepatotoxic models, including chemically induced, drug induced, metal induced, radiation induced, and genetic models, among others. However, a model that directly mimics human hepatotoxicity is required (Liu et al., 2013).

Chemical induced hepatotoxicity

Carbon tetrachloride (CCl_4): Carbon tetrachloride (CCl4) is an inorganic substance. It (CCl4) is a common industrial solvent with high hepatotoxicity (Brautbar and Williams, 2002). It is used in the synthesis to chlorinated organic compounds such as chlortoluorocarbon refrigerants, agricultural fumigants, semiconductor production, fat, oil, and rubber processing, and laboratory applications; it does not occur naturally; it is manufactured chemically to make refrigeration fluid and propellants for aerosol cans (Kauppinen et al., 2000).

Carbon tetrachloride is a common toxicant used to cause experimental liver damage in animals (Brautbar and Williams, 2002). The hepatotoxicity to CCl_4 is mostly caused by its degradation metabolites, trichloromethyl (CCl_3) and trichloromethyl peroxyl (CCl_3O_2), which are produced by the hepatic microsomal enzyme (CYP2E1). These products are unstable radicals with a high propensity for attaching to cell membrane proteins and lipids or abstracting a hydrogen atom from an unsaturated lipid, initiating LPO and causing liver damage.

Thioacetamide: TAA Thioacetamide is a white crystalline solid organosulfur chemical that is soluble in water and is used in the synthesis to organic and inorganic compounds as a source to sulfide ions (Nada et al., 2010). It is commonly used to induce fibrosis and damage both zones 1 and 3 to hepatocytes (Debnath et al., 2013).

Principle: TAA is not toxic to the liver, but thioacetamide-s-oxide, an intermediate metabolite to TAA, covalently binds to hepatic macromolecules, altering cell permeability and increasing intracellular calcium, concentration, resulting in Both zone 1 and zone 3 hepatocytes suffered cellular injury and necrosis (Eidi et al., 2012; Moreira et al., 2014). TAA causes portal-portal or portal central septa formation and cirrhosis at low dosages. TAA therapy required a long time to induce significant fibrosis when compared to other hepatotoxins that increase the danger to premature loss to test animals due to the development to cholangiocarcinoma and hepatocellular carcinoma (HCC) (Shahjahan et al., 2004).

Diethyl nitrosamine (DEN) induced: Diethylnitrosamine is hydroxylated in the liver by CYP2E1 to form ethyldiazonium ion, a bioactive intermediate that causes DNA damage by interacting with nucleophiles, resulting in hepatocyte necrosis in the perivenular and periportal areas with centro-portal fibrotic septa. Low DEN dosages given over time cause HCC. As a result, this model is particularly useful for examining the transition to liver fibrosis to hepatocellular carcinoma (HCC) (Liu et al., 2013).

Drug-induced hepatotoxicity

Hepatotoxicity caused by drugs is either fatal or requires hospitalisation. It could be either predictable or unpredictable. Predictable reactions are toten dose-related and occur in the majority to people who are exposed shortly after a toxicity threshold is achieved. Unpredictable hepatotoxic responses occur without warning, are not dose-related, and have varying latency periods ranging from a few days to months. it involves direct hepatocyte injury by interfering with important cellular activities second broad group is substantially more complex; sensitization to hepatocytes to cytokine-induced damage is one way that directly hazardous or reactive metabolites may generate hepatotoxicity (Palanivel et al., 2008).

Paracetamol: Paracetamol is a widely used analgesic and antipyretic drug and is known to elicit a dose-dependent

effect (Cover et al., 2006). The reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) covalently binds to protein. The formation to paracetamol–glutathione conjugates can lead to irreversible hepatocyte injury and necrosis by varied mechanisms. Binding to NAPQI to glutathione (GSH) sulfydryl groups causing depletion to hepatic anti-oxidative capacity and oxidative damage to numerous cell components ultimately results in necrosis, cell death, and release to the damage-associated molecular pattern to neutrophil (DAMPs). These DAMPs lead to increased production to cytokines (TNF-/IFN-c/chemokines) and platelet-activating factor (PAF) which are putative intracellular mediators and largely responsible for paracetamol hepatotoxicity (Bohmer et al., 2011).

d-Galactosamine and lipopolysaccharides

Lipopolysaccharide is a major constituent to the gram-negative bacteria cell wall and is removed from the systemic circulation largely by Kupffer cells and macrophages in the liver. d-GalN is an amino sugar metabolized by the hepatocytes, induces liver damage, and enhances the production of ROS in hepatocytes. Combination to d-GalN/LPS is an ideal hepatotoxic model that resembles clinical hepatitis, where oxidative stress plays a major role. d-Galactosamine inhibits protein synthesis by depleting the uridine triphosphate pool, causing in early generation to reactive oxygen species and finally apoptosis. Lipopolysaccharide increases the release to proinflammatory cytokines (TNF-), which subsequently induces the increased production to reactive nitrogen species and inflammatory prostaglandins due to the activation to nitric oxide synthase-1 (NOS-1) and cyclooxygenase 2 (COX-2) respectively (Alkiyumi et al., 2012).

Anti-tubercular drugs induced hepatotoxicity: The current anti-tubercular treatment regimens containing isoniazid (INH), rifampicin, and pyrazinamide have the potential to cause significant hepatotoxicity. Multiple drug regimens can occasionally increase the adverse effects of anti-tubercular therapy INH, rifampicin, and pyrazinamide are therefore all potentially hepatotoxic when taken alone, but their toxic effect is increased when given together. Monoacetyl hydrazine is produced from INH, which is then processed by CYP450 to a hazardous compound, resulting in hepatotoxicity. Hepatitis is more common in patients receiving concomitant rifampicin therapy. This is thought to be related to rifampicin-induced CYP450 enzyme upregulation, which causes an increase in the generation to hazardous metabolites from acetyl hydrazine (AcHz). Rifampicin also causes INH to be converted into isonicotinic acid and hydrazine, both to which are hepatotoxic.

INH's metabolite AcHz's plasma half-life is decreased by rifampicin, and by increasing AcHz's oxidative clearance rate, AcHz is quickly converted to its active metabolites. This rapid conversion is related to the higher risk to liver necrosis brought on by the combination to INH and rifampicin**.**

Rifampicin stimulates the hydrolysis pathway to INH metabolism, which results in the hepatotoxic metabolite hydrazine. When rifampicin and pyrazinamide are taken concurrently in tuberculosis patients, pharmacokinetic interactions occur. Pyrazinamide reduces rifampicin blood levels via lowering absorption and increasing elimination. Pyrazinamide appears to be related with an increased incidence to hepatotoxicity when combined with INH and rifampicin (Nafees et al., 2013). In rats the combination to INH and rifampicin at the dose (50 mg/kg, orally) for 28 days produced hepatotoxicity (Kabiri, 2013).

Induced by antibiotics: Antibiotics are substances that produced by microorganisms and frequent uses can selectively suppress or kill the growth to other microorganisms at very low concentrations. They are classified based on the cellular component or system they affect, as well as whether they induce cell death (bactericidal drugs) or simply inhibit cell growth (bacteriostatic drugs)(Park et al., 2009).

Miscellaneous Drugs

Macrolides: Macrolide antibiotics are a well-known cause to cholestatic liver damage. The most prevalent macrolide that causes hepatotoxicity is erythromycin estolate. Other erythromycin formulations, such as propionate, ethyl succinate, and stearate, can, however, induce hepatotoxicity. The predicted risk to erythromycin-associated hepatotoxicity is 3.6 cases per 100,000 people. Symptoms commonly appear 3 to 4 weeks after the initial course to medication and within 2 to 3 days to starting another round to erythromycin. The injury mechanism appears to be immunologic, and the pattern to injury is typically cholestatic. Nausea, vomiting, and stomach discomfort can all be symptoms to acute cholecystitis. A skin rash and eosinophilia are uncommon Erythromycin-induced hepatotoxicity is normally reversible with drug withdrawal within 2 to 5 weeks, but it can last up to 6 months in exceptional cases. Erythromycin is seldom associated with severe lethal liver damage. In 2006, three cases to drug-induced hepatotoxicity were documented with telithromycin, one to which required orthotopic liver transplantation and another to which resulted in death, causing the FDA to issue a black box warning due to hepatotoxicity concerns. Other macrolides, such as clarithromycin, have also been linked to hepatotoxicity.

They inhibits bacterial growth by interfering with their ability to produce proteins, attaching to the 50S ribosomal subunit, and interfering with the translocation to sensitive bacteria. Paracetamol-induced hepatotoxicity mechanism 123 bacteria and thereby interfering with protein production in an animal model to liver disease (Wan et al., 2013).

Anticancer drugs: Cancer is a complex illness characterized by multiple temporo-spatial changes in cell physiology that eventually result in malignant tumors. The disease's biological end point is abnormal cell growth (neoplasia) (Seyfried and Shelton, 2010). Many novel cytotoxic chemotherapeutic drugs have been developed in recent decades to extend the survival to patients with advanced and metastatic malignancies. Recently, particularly targeted antibodies and other biological agents have been used in various combinations with chemotherapy to extend life (Maor and Malnick, 2013). Chemotherapy-induced liver damage is assumed to be due to reactive oxygen species (ROS) formation, which is meant to cause tumour cell apoptosis (Lim et al., 2010).

Principle: Cisplatin is commonly used to treat a wide range of cancer illnesses. The most critical processes implicated in cisplatin-induced toxicity are oxidative stress and drug metabolism. Some proteins and genes are related to drug and fatty acid metabolism, which is also responsible for hepatotoxicity. Elevated CYP2E1 has been shown to increase ROS and oxidative stress, leading to hepatotoxicity (Lawrence et al., 1995).

Hepatotoxicity caused by radiation

Radiation is energy that devices can generate in the form to waves or particle streams. Ionizing radiations (e.g., alpha particles, beta particles, and gamma rays, X-rays) can damage the atoms in living organisms, causing a health risk by harming tissue and DNA in the gene. Another type to radiation is non-ionizing radiation, visible light, and radio waves.

Principle: In radiation injury, central vein endothelium and sinusoidal endothelial cells activate the coagulation cascade, resulting in fibrin build up and clot formation in the central veins and hepatic sinusoids. Hepatic dysfunction may result from the loss to centrilobular hepatocytes and atrophy of the inner hepatic plate. (Lawrence et al., 1995; Dawson et al., 2001).

Metal-induced hepatotoxicity: Induced by mercury Human activities contribute significantly to the pollution to the environment with harmful and carcinogenic metal compounds. Mercury is a transition metal that encourages the development to reactive oxygen species (ROS) like hydrogen peroxides (Singh et al., 2012).

Principle: It promotes the formation to reactive oxygen species (ROS) such as hydrogen peroxides. These ROS encourage the development to lipid peroxides and the highly reactive hydroxyl radical (Miller et al., 1991), Because these lipid peroxides and hydroxyl radicals have the potential to damage the cell membrane, mercury inhibits the free radical-scavenging enzymes catalase, superoxide dismutase, and glutathione peroxidase. The glutathione moiety is reduced in the bile duct and gall bladder to a dipeptide and subsequently to a Lcysteine mercury complex before entering the circulatory system (Benov et al., 1990).

Diet-induced hepatotoxicity

Alcohol: Chronic heavy alcohol consumption results in serious health problems including alcohol liver diseases (ALD). Increased alcohol consumption results in increased release to endotoxin from gut bacteria and membrane permeability to the gut to endotoxin, or both. Elevated levels to endotoxin activate Kupffer cells to release eicosanoids, TNF-α and formation to various free radicals. Prostaglandin increases the oxygen uptake and produces the hypermetabolic state leading to increased oxygen demand causing hypoxia which is further precipitated by regeneration to alpha hydroxyethyl free radicals due to the activation to alcohol dehydrogenase, and ultimately manifest into necrosis to the liver lobule. Such ROS and intermediates bind covalently to proteins, DNA and lipids molecules on cell membrane and damaged tissue and organ. Ethanol also known to affect the immune system and alter

Table 1. Classification to animal models to hepatotoxicity

A. Non-invasive model
a. Chemically induced hepatotoxicity
1. CCl4 induced
2. Thioacetamide induced
3. Dimethyl or diethyl nitrosamine induced
4. Aflatoxin induced
b. Drug-induced hepatotoxicity
1. NSAID induced
2. Anticancer drugs induced
3. Antibiotic induced
4. Anti-TB drugs induced
c. Radiation-induced hepatotoxicity
d. Metal-induced hepatotoxicity
1. Mercury induced
e. Diet-induced hepatotoxicity
1. Alcohol-induced
2. High-fat diet-induced

cytokine production leading to increased hepatic triglycerides, LPO and depleting hepatic glutathione (GSH) content. In Lieber–DeCarli liquid diet (LDLD) model, alcohol feeding is insufficient to cause liver injury unless, a dextrin and maltose mixture supplemented with fats, essential vitamins, minerals and fibres. Long-term administration to alcohol (four week) on the LDLD leads to microsomal enzyme (CYP2E1) induction, formation to ROS, increased triglycerides, inflammatory cell infiltration, changes in iron homeostasis/anemia, and nutritional deficiencies that finally manifest in liver damage. In Tsukamoto French (TF) intragastric feeding model, rats were fed with alcohol directly through a surgically implanted intragastric cannula. Alcohol intake is administered by a liquid diet at a defined rate over a specified time period. The model showed higher blood and urine alcohol content than animals fed with alcohol using the LDLD method or alcohol in drinking water. The alcohol in the drinking-water method involves feeding to alcohol in drinking water is gradually increased from 10 to 40% (v/v) with standard rat diets ad libitum. In this model significant hepatic steatosis and inflammation development take place without fibrosis and cirrhosis (Deepa and Ingawale, 2013).

High fat diet (NAFLD): Non-alcoholic fatty liver disease (NAFLD) refers to a histological spectrum to liver disease caused by obesity, diabetes, and insulin resistance, ranging from isolated steatosis to steatohepatitis and cirrhosis. NAFLD/ NASH pathogenic hypotheses must account for the substantial linkages between overnutrition and underactivity, insulin resistance, and hereditary variables (Adams et al., 2005). Lipotoxicity, oxidative stress, cytokines, and other pro-inflammatory mediators may all contribute to the progression to steatosis to NASH. Changes in socioeconomic situations and accompanying changes in food intake, food composition, and physical activity (together referred to as "lifestyle") may all have a role in NASH (Musso et al., 2003).

Principle: Non-alcoholic fatty liver disease (NAFLD) refers to a type to liver disease that includes steatosis (fatty change). The liver is important in lipid metabolism because it imports serum-free fatty acids and builds them up, as well as stores and exports lipids and lipoproteins. Fatty liver is defined by an excess accumulation to lipids, primarily triacylglycerols (TAG), in hepatocytes, which is classified according to the size to the lipid vacuoles, macro-vesicular or micro-vesicular steatosis (Adams et al., 2005), dyslipidemia, insulin resistance (IR), and type II (non-insulin-dependent) diabetes mellitus are all closely linked to NAFLD.

Procedure: The animals were housed in pairs and had unlimited access to food and water. Light (12-hour light-dark cycle), humidity, and room temperature were all adjusted in their surroundings (20–23C). For 8 weeks, all animals were randomly assigned to a standard (SD) or high-fat (HF) diet (Ludwig et al., 1980).

Conclusion

The main cause to hepatotoxicity is the production to free radicals, inflammatory markers, hereditary impairment, and other factors that result in various pathological alterations. Hepatic stellate cells, hepatotocytes, and Ito cells, on the other hand, are directly and indirectly impacted by hepatotoxicity. Their excessive activity indicated another method to liver harm caused by any toxicant. Rodents have similar physiology to humans, which makes rodent animal models useful in understanding the pathogenesis and aetiology to hepatotoxicity. There are multiple causal substances that cause liver damage through various processes, such as CCl_4, alcohol, a high fat diet, and so on. The focus to this review is on animal models and their causal agents, as well as what type to degenerative alteration causes hepatotoxicity. In the present review, our main emphasis is on animal model and their causative agent and what type to pathological change induces hepatotoxicity.

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