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RECENT ADVANCES IN HEPATOTOXICITY STUDIES

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ABSTRACT: *Hepatotoxicity has many facets. Those to be discussed in this review include the mechanism for the hepatotoxic effects, nature of the injury, and animal models of hepatotoxicity suitable for the detection of chemical injury. Some therapeutic drugs used for treatment of hepatitis are also presented. In addition, as an important and serious problem in future, alternative toxicity testing is discussed.*

INTRODUCTION

Liver injury induced by chemical agents has been recognized as a toxicological problem in past 100 years. The lesion observed depends not only on the chemical agent involved but also on the period of exposure. In a case of acute exposure, several toxicological events, *i.e.*, lipid accumulation in the hepatocytes, cellular necrosis, or hepatobiliary dysfunction are observed. Whereas cirrhotic or neoplastic changes are usually considered to be the result of chronic exposures. Some forms of liver injury are reversible while others result in a permanently deranged organ. In addition to chemical-induced hepatotoxicity, viral hepatitis is also serious problem in recent years since Blumberg first reported Australia antigen in 19 65.

The purpose of this review is to summarize the outline of liver injuries and recent advances in knowledge of hepatotoxicity.

1. Susceptibility of liver

The liver is often the target organ for injuries induced by chemicals and hepatitis viruses, and several important factors are known to contribute to the susceptibility of liver. First, most xenobiotics enter the body through the gastrointestinal tract (gut) and, after absorption, are transported by the hepatic portal vein to the liver, while hepatitis virus enters the body by oral route or transfusion.

A second factor is a high concentration in the liver of drug metabolizing enzymes. Biotransformations of xenobiotics consist of two successive steps such as phase 1 reaction (oxidation, reduction, hydrolysis) and phase 2 reaction (conjugation), and many phase 1 reactions produce reactive metabolites which can induce lesions within the liver. In most cases, areas of damage are in the centrilobular region, and this localization has been attributed to the higher concentration of the enzymes producing reactive metabolites in that area of the

Table 1. Examples of acute hepatotoxic chemicals

Chemical	Injuries	Chemcial	Injuries
Acetaminophene	N	Ethionine	F
Acetylaminofluorene	C	Furosemide	N
Allyl alchol	N	Galactosamine	N,F
Allyl formate	F	Methotrexate	F
Aflatoxin	N,F,C	Mitomycin C	F
Azaserine	N,F	Phosphorus	N,F
Bromobenzene	N	Polychlorinated	
Bromotrichloromethane	N,F	biphenyls	C
Carbon tetrachloride	N,F	Puromycin	F
Chloroform	N,F	Pyrrolizine	
Cycasin	C	alkaloid	N,F,C
Cycloheximide	F	Safrol	
Dimethylaminoazobenzene	N,F,C	Tannic acid	N,F
Dimethylnitrosamine	N,F,C	Tetrachlorethylene	N,F
Emtine	F	Tetracycline	F
Ethanol	F	Thioacetamide	N
		Trichloroethylene	N,F
		Urethane	N,C
		Vinyl chloride	C

N, Necrosis; F, Fatty liver; C, Carcinogenesis (in experimental animals)

Table 2. Examples of drugs which induce intrahepatic cholestasis

Ajimaline	Methylandrostenolone
Amitriptyline	Methyltestosterone
Azathioprine	Methylthiouracil
Carbamazepine	Oxacillin
Carbarsone	Penicillamine
Chlordiazepoxide	Perphenazine
Chlorpromazine	Prochlorperazine
Chlorpropamide	Promazine
Chlorthiazide	Propoxyphene
Diazepam	Propylthiouracil
Erythromycin	Thioridazine
Estradiol	Thiouracil
Ethacrynic acid	Tolazamide
Fluphenzaine	Tolbutamide
Haloperidol	Triacetyloleandomycin
Imipramine	Triflupromazine
17-Methylnortestosterone	

liver.

2. Classification of chemical-induced liver injury and mechanisms for hepatotoxicity

There are a veriety of classifications of liver lesions induced by various chemical

agents. In addition to acute hepatic necrosis and fatty liver (Table 1), the drugs producing cholestatic type of response are also recognized (Table 2). This latter lesion results in decrease or cessation of bile flow, and, thereafter, retension of bile salts and bilirubin occurs. These changes in liver function lead to production of jaundice. The third one is a type of chemical-induced hepatitis which resembles closely that produced by viral infections. The drugs associated with this lesion are shown in Table 3. A number of drugs are also associated with a mixed type of lesion, that is, these drugs possess both cholestatic and hepatocellular components (Zimmerman, 1978).

Injury of the cells can be initiated by a number of mechanisms, *i.e.*, inhibition of enzymes, depletion of cofactors, and alteration of cell membranes. Many compounds, including clinically useful drugs, cause cellular damage through metabolic activation of the chemical to reactive metabolites, and these reactive metabolites may bind covalently to cellular macromolecules such as nucleic acids, proteins, lipids, and polysaccharides, thereby changing their biological properties.

The activation reactions are catalyzed by the drug metabolizing enzymes such as cytochrome P-450, and pretreatment with enzyme inducers increases the formation of the reactive metabolites of the some chemical agents.

Conjugation reactions of the reactive metabolite usually exist within the cell for the rapid removal and inactivation of many potentially toxic compounds. Thus, cellular toxicity depends primarily on the balance between the rate of formation of reactive metabolites and the rate of their removal.

3. Type of liver injury

1) Fatty liver

Some chemical agents that produce liver injury cause an abnormal accumulation of triglycerides, in the parenchymal cells. Although many toxicants cause lipid accumulation in the liver, the mechanisms may differ. Namely, excess lipid can result from oversupply of free fatty acids from adipose tissues, or more commonly, from impaired release of triglycerides as very-low-density lipoprotein(VLDL) from the liver into the plasma.

Table 3. Examples of drugs which induce hepatitis

Acetohexamide	Methoxyflurane
p-Aminosalicylic acid	Alpha-Methyldopa
Carbamazepine	Nialamide
Cinchophen	Papaverine
Dantrolene	Phenylbutazone
Ethacrynic acid	Pyrazinamide
Ethionamide	Sulfamethoxazole
Halothane	Sulfisoxazol
Ibufenac	Tranylcypromine
Indomethacin	Trimethobenzamide
Imipramine	Zoxazolamine
Isoniazid	
6-Mercaptopurine	

2) Cell necrosis

Cell necrosis or cell death is usually an acute injury and may be focal(central, midzonal, or peripheral) or massive. Because of the regenerating capability of the liver, necrotic lesions are not necessarily critical. Cell death occurs, along with rupture of the plasma membrane, and is preceded by a number of morphological changes such as cytoplasmic edema, dilation of endoplasmic reticulum, and swelling of mitochondria with disruption of cristae. Biochemical events include lipid peroxidation and subsequent membrane destruction, and disturbance of cellular Ca^{2+} homeostasis.

3) Cholestasis

The stoppage or suppression of the flow of bile causes cholestasis and may have either intrahepatic or extrahepatic causes. Inflammation of the bile ducts results in retention of bile as well as bilirubin accumulation, leading to jaundice.

4) Hepatitis

Hapatitis or inflammation of the liver, is usually viral in origin; however, certain drugs can induce a liver injury which closely resembles viral hepatitis. This type of liver injury is very difficult to demonstrate in laboratory animals and is often manifest only in susceptible individuals.

5) Cirrhosis

This progressive disease is characterized pathologically by the presence of collagen throughout most of the liver. Cirrhosis is often associated with liver dysfunction, frequently resulting in jaundice. In humans, chronic use of ethanol is the single most important cause of cirrhosis. Ethanol-induced hepatitis is detailed elsewhere in this article.

6) Carcinogenesis

The most common type of primary liver tumor is hepatocellular carcinoma; other types include cholangiocarcinoma, angiocarcinoma, glandular carcinoma, and undifferentiated liver cell carcinoma. Although a wide variety of chemicals are known to induce hepatoma in laboratory animals (Table 1), only a few chemicals are known to be human carcinogens.

4. Some examples of the agents producing liver injury

Problems with therapeutic drugs occur due to accidental acute intoxication by children and deliberate suicidal and homicidal overdose by adults.

1) Acetaminophen

Acetaminophen (APAP) is a widely used analgesic which is normally safe when taken in therapeutic doses, but an overdose may cause acute centrilobular hepatic necrosis, which can be fatal. Although APAP is eliminated primarily by formation of glucuronide and sulfate conjugates, a small proportion is metabolized by cytochrome P-450 to produce a reactive electrophilic intermediate (NAPQI, N-acetyl-p-benzoquinone imine) (Fig. 1). This reactive intermediate is labile and usually inactivated by conjugation with glutathione and excreted. Higher doses of APAP will progressively delete hepatic glutathione levels, however, resulting in extensive covalent binding of the reactive metabolite to liver macromolecules with subsequent hepatic necrosis. In addition to covalent binding, mitochondrial dysfunction such as failure of the cell respiration has been recognized. More

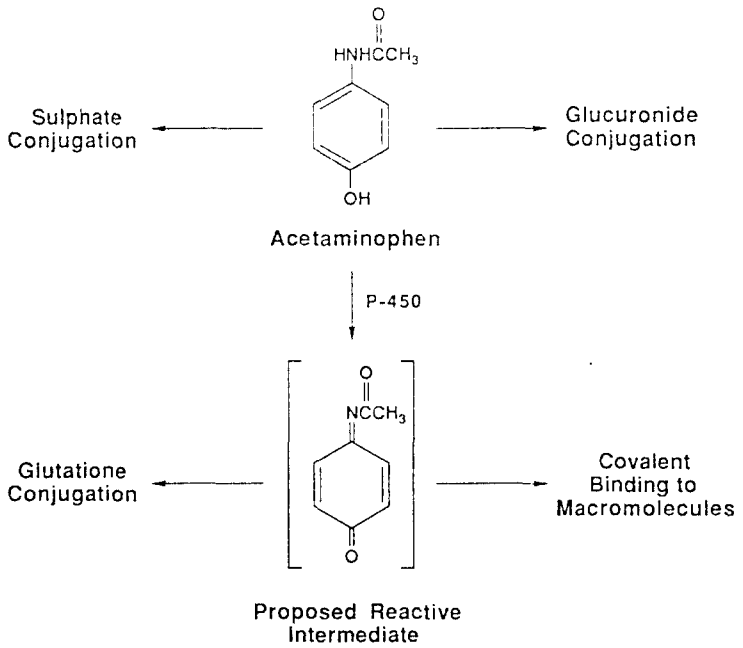

Fig. 1. Metabolism of acetaminophen showing formation of a reactive metabolite.

recently, Holme *et al.* (1991) reported that N-acetyl-m-aminophenol, a regioisomer of APAP, showed approximately 10-fold less toxicity than APAP.

2) Isoniazid

Therapeutic doses of isoniazid lead to liver injury in some patients. This damage is due to a reactive intermediate formed from the isoniazid metabolite, acetylisoniazid (Fig. 2). Liver damage induced by isoniazid occurs most often in patients who are genetically fast acetylators, namely, they metabolize isoniazid more rapidly than do slow acetylators. Among both black and white Americans, about 50% of the population are fast acetylators. The Japanese are also rapid acetylators, with about 88% of the population. This is a good example of pharmacogenetic studies.

3) Halothane

Halothane is known to cause both a mild and a severe form of hepatotoxicity in patients. The milder form of hepatotoxicity is characterized by minor elevations in serum transaminase levels and has been reported in about 20% of patients anesthetized with halothane. The severe form of hepatotoxicity, so call halothane hepatitis of fulminant one, however, is often fatal and much rarer. The liver damage is often centrilobular, and a considerable degree of hepatic necrosis.

Halothane is metabolized in the liver by cytochrome P-450-mediated oxidation and reduction (Fig. 3) to two chemically reactive metabolites, trifluoroacetyl (TFA) halide and 1-chloro-2,2,2-trifluoroacetyl radical(Gandolfi *et al.*, 1980). The TFA group forms the neoantigen by covalent binding to microsomal protein. The patients antibodies recognize epitopes that consist of a combination of the TFA

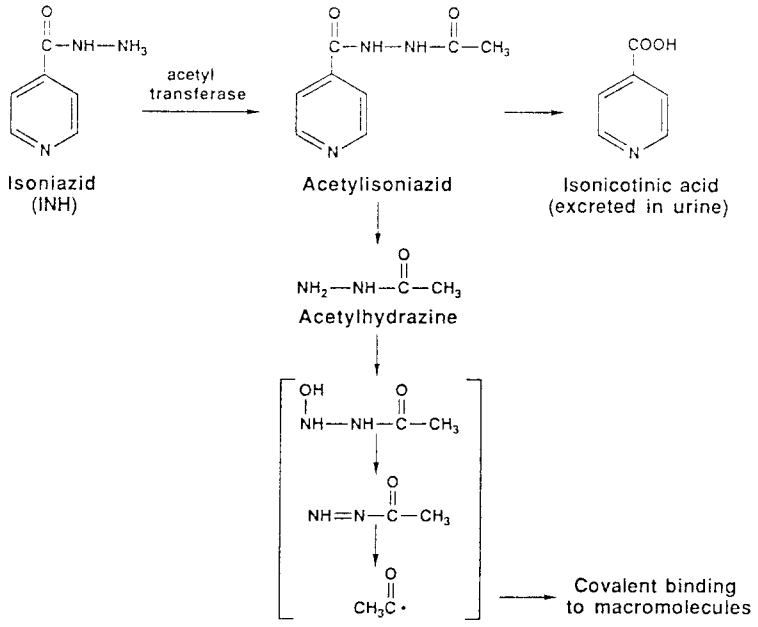

Fig. 2. Metabolism of isoniazid showing formation of a reactive metabolite.

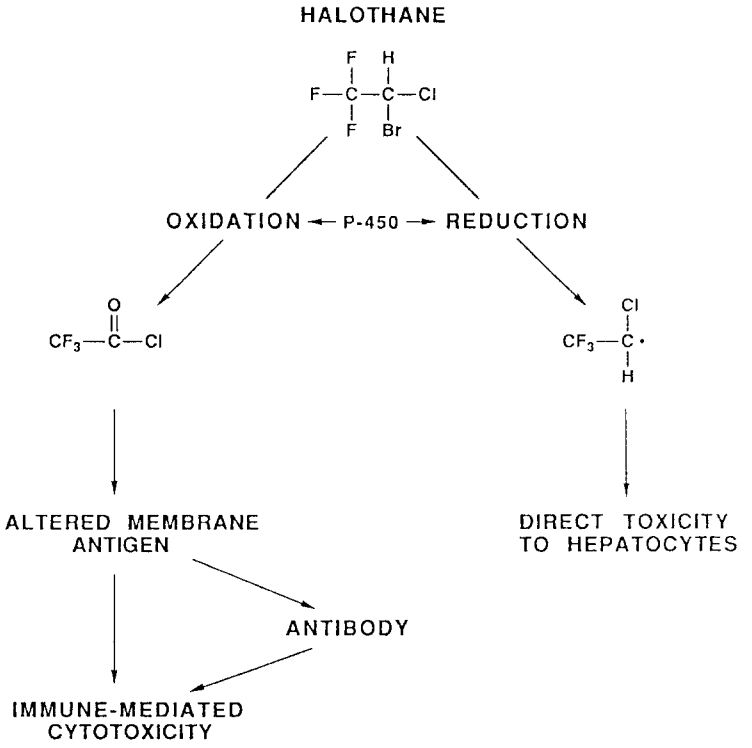

Fig. 3. Metabolism of halothane by the reduction and oxidation pathways.

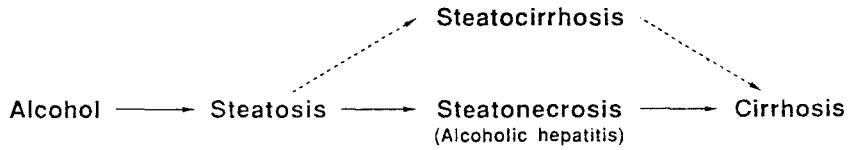

Fig. 4. Histogenesis of cirrhosis due to alcoholism.

group together with unique structural determinants of the polypeptide carrier. The microsomal protein which is bound to TFA group of halothane metabolite was named 59 kDa which is an isozyme of liver microsomal carboxylesterases (Pohl *et al.*, 1989; Satoh *et al.*, 1989). Later, the isozyme involved was characterized as RH1 (Hosokawa *et al.*, 1990). Studies in both animals and humans have suggested that all individuals exposed to halothane generate the antigen and that, therefore, if hepatitis is immune mediated, the defect in susceptible patients is more likely in the immune response to antigen, rather than in metabolic antigen generation.

4) Ethanol

The liver disease seen in alcoholics encompasses three main related entities: steatosis, alcoholic hepatitis, and cirrhosis. In addition, hemochromatosis and hepatic carcinoma are seen in association with alcoholism. Steatosis is the initial histologic manifestation of alcoholic liver disease, and cirrhosis is the terminal lesion. Alcoholic hepatitis appears to be a stage in the development of cirrhosis (Fig. 4).

Alcoholic hepatitis is induced by the formation of acetaldehyde-protein complex in the liver. Acetaldehyde, the primary metabolite of ethanol, reacts with proteins to give both stable and unstable adducts (Sorrell and Tuma, 1987). Lieber and DeCarli (1970) and Ohnishi and Lieber (1977) reported that ethanol was oxidized by the microsomal ethanol oxidizing system (MEOS) which is one of the enzymes responsible for the generation of acetaldehyde. Later, MEOS was identified as cytochrome P-4503a in rabbits (Morgan *et al.*, 1982), P-450J in rats(Koop *et al.*, 1984; Ryan *et al.*, 1986) and P-450 H_j in human (Wrighton *et al.*, 1987).

On the other hand, the major stable adducts formed from acetaldehyde and hemoglobin *in vitro* were shown to be 2-methylimidazolidin-4-ones formed by reaction at the N-terminal valine residues of peptide chains, particularly the B-chains (San George and Hoberman, 1986). There is much interest in the formation of such conjugates because of their possible relevance to the short- and long-term effects of alcohol injestion.

5) Hepatitis viruses.

Viral hepatitis is classified into six categories in terms of the virus involved (Table 4). Among these hepatitis, chronic type B and type C hepatitis are most important and serious form of chronic liver disease. Approximately 200 million persons worldwide are chronic carriers of hepatitis B surface antigen(HBsAg), and an appreciable proportion of these individuals have chronic liver disease. Although these HBsAg carriers are at a high risk of developing cirrhosis and hepatocellular carcinoma, there is still no specific therapeutic drug for chronic type B hepatitis.

Table 4. Nomenclature of viral hepatitis and hepatitis viruses

Nomenclature			
Old		New	
Disease	Virus	Disease	Virus
Type A hepatitis	HAV	Type A hepatitis	HAV
Type B hepatitis	HBV	Type B hepatitis	HBV
NonA, NonB type hepatitis*	NANAV	Type C hepatitis	HCV
Delta hepatitis	Delta Virus	Type D hepatitis	HDV
Epidemic endemic, enterically -transmitted NonA, NonB type hepatitis**	ENANB	Type E hepatitis	HEV

*NonA, NonB type hepatitis virus after transfusion (parenteral).
**New hepatitis virus which is differentiated from the NonA, NonB virus after transfusion.

Recently, vaccine for type B hepatitis was developed and is found to be very useful for treatment of patients suffered from type B hepatitis.

In 1990, Reyes *et al.* have characterized the major agent of enterically transmitted nonA, nonB hepatitis, termed the hepatitis E virus (HEV) through molecular techniques similar to those used in the characterization of the hepatitis C virus(HCV) (Choo, Q.-L. *et al.*, 1989). On the other hand, Bradley *et al.* (1990) used an established chimpanzee model of parenterally transmitted nonA, nonB hepatitis in order to define virus-specific immune response patterns in acutely and persistently infected animals. Serial bleeding were obtained from 23 chimpanzees that had been experimentally infected with an isolated of hepatitis C virus.

With regard to the relationship between development of hepatitis B and production of lymphokine, Anastassakos *et al.* (1988) have simultaneously assayed interleukin-1 and interleukin-2 production in 31 chronic carriers of the hepatitis B virus in order to determine whether the abnormalities of lymphocyte proliferation in chronic hepatitis B virus infection may be secondary to disordered lymphokine production. The results indicate that interleukin-1 production was markedly elevated in patients with chronic hepatitis B virus infection, whereas in contrast, interleukin-2 production was found to be reduced in these patients. There was a highly significant correlation between interleukin-1 production and the severity of fibrosis, suggesting that this lymphokine may be closely related to the development of cirrhosis in such patients.

5. Animal models of hepatitis

1) Chemical-induced liver injury

(1) Treatment of the animals with chlorinated hydrocarbons.

Carbon tetrachloride and other chlorinated hydrocarbons are known to induce hepatic injury in experimental animals. Mechanisms involved in this hepatotoxicity

are (1) free radical formation, (2) lipid peroxidation, and (3) covalent binding.

(2) Ethionine, galactosamine and choline-deficient diet.

Like carbon tetrachloride, ethionine and galactosamine are widely used to produced hepatic injury. Choline-deficient diet is also used to make hepatotoxic animals. Certain dietary modifications lead to alterations in cells increasing their susceptibility to carcinogens. Thus, choline deficiency in rats has been shown to enhance the induction of preneoplastic foci as well as hepatoma by well-known carcinogens. A choline deficiency leads to damage to DNA followed by cell death and cell proliferation.

2) Woodchuck

The human Hepatitis B virus (HBV) is a member of a family of viruses known as hepadnaviruses. Other viruses in this family are the woodchuck hepatitis virus (Summer *et al.*, 1987), the ground squirrel hepatits virus (Marion *et al.*, 1983), and the duck hepatitis B virus (Mason *et al.*, 1980; Marion *et al.*, 1987). These animal viruses have been invaluable models for characterization of hepadnaviruses and delineation of their unusual replicative cycle. Up to 1987, considerable informations concerning the morphology of the human hepatitis B virus and its genome structure have been accumulated. Among these reports, Summers *et al.* (1987) demonstrated that woodchuck hepatitis virus is a second member of a novel class of viruses presented by the human hepatitis B virus. Thereafter, hepatitis in woodchucks has been widely used as an experimental system for investigation of the oncogenic potential of hepatitis B virus-like virus.

3) Hereditary hepatitis

LEC rats (Long-Evans rats with a Cinnamon-like coat color), an inbred strain that has been separated from Long-Evens rats, spontaneously develop acute hepatitis about 4 months after birth. The clinical signs of the hepatitis resemble those of human fluminant hepatitis (Yoshida *et al.*, 1987). Genetic analysis has revealed that a single autosomal recessive gene is responsible for the hepatitis. Recent papers reported that in LEC rats an abnormal copper metabolism may be maintained during the process of hepatic carcinogenesis (Yu Li *et al.*, 1991; Ono *et al.*, 1991; Sugawara *et al.*, 1991).

6. Therapeutic drugs for hepatic injury

1) Interferon: Although α-interferon has shown great promise in a subset of patients treated for prolonged periods, the response rates overall have unfortunately been disappointingly low. Hoofnagle *et al.* (1988) conducted a prospective, randomized, controlled trial of recombinant α-interferon in 45 patients with chronic type B hepatitis. It was concluded that a 4-months course of the drug treatment can induce a remission in disease in approximately one-third of patients with chronic hepatitis B. In 1987, efficacy of interferon α2a for hepatitis type C was approved in Japan. This drug is effective for treatment of HCV(hepatitis C virus) with decrease of transaminase activity in 60-70% of total number of patients, and HCV in 30-40% of total patients treated was eliminated. Interferon causes some side effects such as depression and fever.

A controlled, randomized trial of a short-term, medium-dose combination

therapy of β- and γ-interferon was performed for 16-24 months in 20 patients with chronic active hepatitis B. The results of this study suggest that a combination of β- and γ-interferon may be an effective therapy for chronic hepatitis B when started early after infection (Caselmann *et al.*, 1989).

2) Nucleic acid analogues:
Adenine arabinoside(Bassendine *et al.*, 1981), adenine arabinoside monophosphate (Garcia *et al.*, 1987), and acyclovir (Schalm *et al.*, 1985; Alexander, 1986) have been used with only limited success in treating this disease. Recently, Kassianides *et al.* (1989) reported that 2′, 3′-Dideoxycytidine, a potent antiviral agent, inhibits the reverse transcriptase of the human immunodeficiency virus *in vitro*. This compound was assessed in 16 Pekin ducks chronically infected with the duck hepatitis B virus. Dideoxycytidine has potent antiviral activity against duck hepatitis B virus and warrants further evaluation as an antiviral agent in the treatment of chronic hepatitis B virus infection in humans.

3) Glutathione: Reduced glutathione is well recognized to play an important protective role by trapping electrophilic metabolites and preventing their binding to hepatic proteins and enzymes. Glutathione conjugation usually results in the formation of a nontoxic, water-soluble metabolite which is then easily excreted.

4) N-Acetylcysteine: Bruno *et al.* (1988) reported that the post-arylative mechanisms by which N-acetylcysteine (NAC) reduces the severity of the hepatotoxicity induced by acetaminophen in primary cultures of mouse hepatocytes. The data suggest that the post-arylative efficacy of NAC may reside in the ability of the antidote to restore the functional capacity of the proteolytic system to rid the cells of arylated proteins. Park *et al.* (1987) also reported that NAC is utilized by the hepatocytes to facilitate the resynthesis of glutathione which is effective for protection of hepatotoxicity.

5) Traditional medicines
(1) Aucubin: Change *et al.* (1985) extracted the pharmacologically active component, named Aucubin from some oriental medicines, *P. asiatica* seed and *A. japonica* leaves. Aucubin has a potent protective effect against liver damage induced by carbon tetrachloride and showed a significant protective activity against α-amanitin poisoning. Also, Aucubin was found to have an inhibitory effect on RNA biosynthesis in liver.
(2) Xiao-Chai-Hu-Tang(XCHT) (Syō-Saiko-tō): Oral administration of XCHT, a boiling extract of a mixture of seven herbs, attenuated the hepatic fibrosis developed in mice after repeated doses of carbon tetrachloride and d-galactosamine. Pretreatment of mice with XCHT reduced the derangement of liver function test seen after a single dose of hepatotoxicants. XCHT may be effective in the treatment of chronic liver injury through protection of hepatocytes (Amagaya *et al.*, 1988; Amagaya *et al.*, 1989).

7. Alternative toxicity testing

Of the *in vitro* hepatic models available to investigate the metabolism and toxicity of xenobiotics, the following preparations have been utilized the most

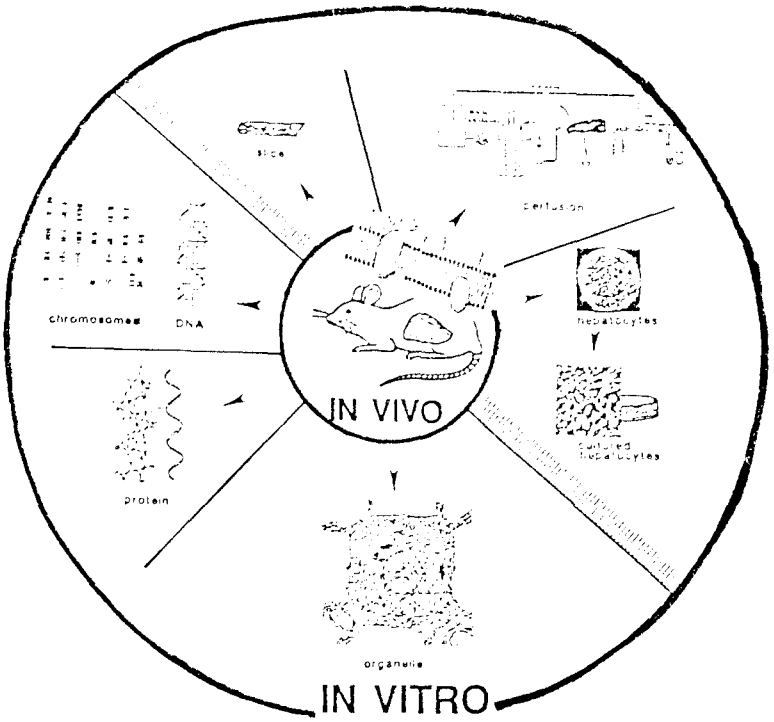

Fig. 5. *In vitro* toxicity testings alternative to *in vivo* experiments.

Table 5. *In vitro* liver systesm used for cytotoxicity and metabolism studies

System	Advantages	Disadvantages
Perfused liver	Retention of structural integrity; Maintenance of cell-to-cell communication.	Short viability period of a few hours; complex and costly perfusion apparatus.
Liver cell lines	Increased viability period; easier to maintain than primary cultures.	Loss of differntiated liver functions; characteristics of cancer or transformed cells.
Freshly isolated hepatocytes	Ease of isolation: comparable drug metabolizing activity as intact liver; ability to evaluate toxicity and metabolism of xenobiotics in same system.	Lack of cell-to-cell contact; short viability period(a few hours); damage to membranes by isolation procedures: Leakage of cofactors and enzymes; impaired intermediary metabolism.
Primary hepatocyte culture	Increased longevity over perfused liver and isolated hepatocytes; recovery from trauma and damage of isolation procedure; retention of several differentiated liver functions; capability for use in conduction of acute and chronic toxicity and metabolism studies of xenobiotics.	Loss of cytochrome P-450 with time in culture; loss of several differentiated functions upon subculturing the cells

extensively: perfused liver, liver cell lines, isolated hepatocytes in suspension, and primary cultured hepatocytes (Fig. 5). Table 5 summarizes the advantages and disadvantages of the various liver models. On the basis of this comparison, we have used a primary culture system of rat hepatocytes to evaluate the cytotoxicity and metabolism of xenobiotics. In these studies, a number of parameters that are considered characteristic of functional hepatocytes are normally used. These include glutathione conjugation, cytochrome P-450 content, aminopyrine N-demethylation, metabolic activation of xenobiotics to toxic intermediates, and covalent binding level. This toxicity testing system is more reliable in predicting the likehood of liver injury *in vivo* than are those using other models as outlined.

In this review, some alternative toxicity testings which have been newly developed in our laboratory are presented.

1) Liver microsomal carboxylesterase as an excellent marker of hepatotoxicity.

As an alternative toxicity testing of hepatotoxicity, Satoh *et al.* (1991a, 1991b) reported that rat liver microsomal carboxylesterase is susceptible to hepatotoxicants including pesticides when added test compounds to the cultured hepatocytes. In the cultured hepatocytes, microsomal carboxylesterase activity is more susceptible and more stable compared to the changes in cytochrome P-450 content.

Recently, Maki *et al.* (1991) reported that among the three major carboxylesterase isozymes, RL1, RL2 and RH1, present in microsomes from rat liver, RL2 shows hydrolyzing activity towards 12-O-tetradecanoylphorbol-13-acetate and 1-oleoyl-2-acetyl-*rac*-glycerol, both activators of protein kinase C. Since protein kinase C has been suggested to be involved in carcinogenesis and cell proliferation, alterations in hepatic microsomal esterase isozymes including RL2 were studied during hepatocarcinogenesis induced by the Solt-Farber model. The isozyme composition of hepatic microsomal carboxylesterase was changed after partial hepatectomy, and marked decreases in RL2 activity was observed at 4 weeks, at the time of preneoplastic foci induction. These findings suggest that RL2 may be involved in regulation of protein kinase C activity by metabolizing its activators at an early stage of hepatocarcinogenesis in rats.

2) Release of liver microsomal β-glucuronidase from hepatocytes *in vitro* and *in vivo* by hepatotoxicants and pesticides.

Release of β-glucuronidase from egasyn-β-glucuronidase complex in the liver microsomes into the culture medium of hepatocytes is a good marker of the hepatotoxicity. Liver microsomal β-glucuronidase is stabilized within microsomal vesicles by complexation with the protein, named egasyn, which is one of the carboxylesterase isozymes (Medda *et al.*, 1986). The egasyn-β-glucuronidase complex is easily dissociated, resulting β-glucuronidase is released to blood *in vivo* or into the cultured medium *in vitro* after administration *in vivo* or *in vitro* addition of hepatotoxic agents or pesticides (Satoh *et al.*, 1991a, 1991b). We conclude that the release of liver microsomal β-glucuronidase is the most rapid and sensitive marker to pesticide- or chemical-induced hepatotoxicity.

3) Multicellular Spheroids

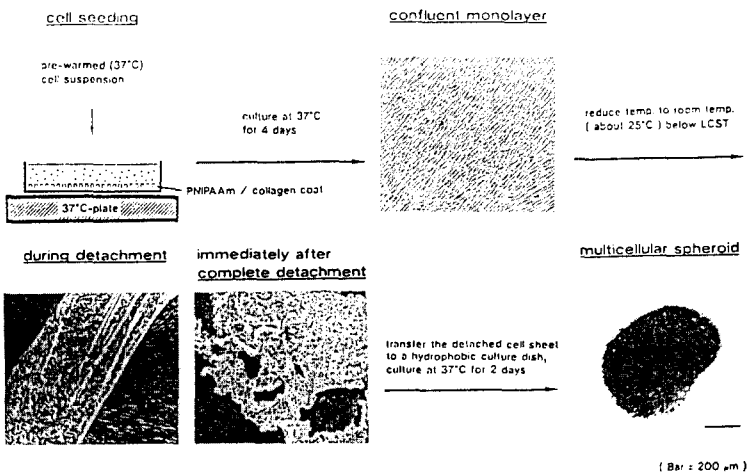

Fig. 6. The scheme of a multicellular spheroid formation process using the substratum composed of PNIPAAm hybridized with type-1 collagen. (PNIPAAm: Poly-N-isopropyl acrylamide; thermoresponsive polymer)

Takezawa *et al.* (1990, 1992) have developed a culture substratum composed of type-I collagen and a thermoresponsive polymer, poly-N-isopropyl acrylamide, which is insoluble in culture medium at 37℃ and becomes soluble below the LCST (lower critical solution temperature: about 30℃), and established a new spheroid formation method by harvesting confluent cells on the substratum as a sheet merely by lowering the temperature to below the LCST. Overall techniques are shown in Fig. 6. This technique offers a reversible transition between two-demensional monolayered cells in the proliferation stage and three-demensional multicellular spheroids in the dormant stage. Recently, we succeeded in making hetero-spheroids composed of mesenchymal cells and epithelial cells by co-culturing them on the above mentioned substratum using a similar method(Satoh *et al.*, in preparation). This hetero-spheroid, a model of organoids(organ-like construction) which seemingly shows the tissue/organ-specific functions, and the above mentioned transition state, are both expected to be used for a new type of toxicity testing(Satoh, *et al.* 1991b).

CONCLUSION

A wide variety of natural and synthetic compounds can damage the liver as a result of varying types of exposure. The mechanism for injury may be intrinsic toxicity of the agent, a particularly susceptible(idiosyncratic) host or a mixture of the two. There are two main type of idiosyncrasy, hypersensitivity and metabolic idiosyncrasy. Chemical agent-induced hepatic injury may be acute or chronic. Acute injury may be mainly cytotoxic or cholestatic. The forms of cytotoxic injury are necrosis, steatosis, or both. Cholestatic injury may be associated with portal inflammation, or may not be. The chronic injury includes cirrhosis, steatosis, and a number of other lesions.

On the other hand, a wide variety of procedures have been used to detect and study toxic injury. The use of *in vivo* models has involved selection of parameters from among the many available for the detection of liver damage in the intact animal. *In vitro* models have been used largely for the study of possible mechanisms of injury, but offer promise as a means of identifying potential drug toxicity.

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