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Models and Markers: *In vitro* and *in vivo* Liver Toxicity Studies

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ABSTRACT

Advances in modern medical sciences concerning hepatic ailments are found inadequate and so different single and polyherbal preparations are being tested in every country for general heaptic health of public. In almost all *in vivo* studies for testing the hepatoprotective formulations or crude juices/extracts, prevalently kidney function tests are carried along with liver function tests due to the close metabolic proximity of these distant organs. Present article summarizes various toxicity models and relevant tests preferred by the researchers during hepatoprotective studies.

INTRODUCTION

As the largest organ of body, liver has a major role in maintenance, performance and homeostasis of the body. Liver provides a chief site for many biochemical pathways concerned with digestion, growth, nutrient and energy supply as well as disease resistance. Chronic liver diseases are the fifth most frequent cause of death in the European Union, as they entail multiple risks, such as portal hypertension, ascites, spontaneous bacterial peritonitis, hepatorenal and hepatopulmonary syndromes, hepatic encephalopathy and, of course, hepatocellular carcinoma (HCC)[1]Both in vitro and in vivo methods are utilized in the liver toxicity studies.

In vitro studies

Isolated liver cells, liver slices, and isolated organs (perfusion or *in situ*) are conventionally well known in vitro models to study hepatotoxicity. [2]*In vitro* models are applied at various levels of research [3-5]

Isolated liver cell models: Cultured liver cells represent the most frequently used in vitro liver cell model. Apart from experimental models, even human cultured hepatocytes have also been established. Currently the modified protocol of 2-step collagenase technique works well for short term cultures of hepatocytes of variety of species. For longer duration models, different approaches are considered viz., addition of basement membrane, culture in collagen sandwich or co-culture with epithelial cells.

Isolated perfused organs: Rat model of isolated perfused liver has been used in a variety of studies for the investigation of drug and chemical-induced hepatotoxicity. The organ perfusion studies can be

conducted with autologous blood or a blood free perfusion. It's a complex model where it's difficult to keep organ function within physiological ranges.

Precision Cut Liver Slices (PCLS) : As the cell culture models miss cell-cell interactions and isolated perfused organ models are too complex, PCLS retain tissue organization and cell-cell matrix interactions. However bile flow and functional parameters suchas portal flow cannot be analyzed. This models can be used for periods of 2 to 3 days.

***In vitro* models**

Advanced in vitro methods involve 2D monolayer culture of cells[6], Collagen sandwich. The current *in vitro* trends involve 3D bioreactors, multicellular spheroids and microdevices or chips.[7]

Cell types in in vitro studies:

Primary hepatocytes of rodents[6]or humans remains a general choice to study the hepatocytes in vitro. However hepatic cancer cell lines as well as hepatocyte like cells obtained from stem cells are also used in the *in vitro* methods.[7]Numerous cell types used *in vitro* are verily classified by Zhang *et al*[8]as immortalized cell lines, transfected cell lines, hepatocytes and membrane vesicles grouped under title 'in vitro transporter models'.

***In vitro* metabolic models**

Different metabolic models used *in vitro* are further classified as Expressed enzymes, sub-celluar fractions and whole cell systems [8]

In vivo Studies

Study Durations:

OECD guidelines recognize acute, sub-chronic and chronic durations of toxicity studies. The guidelines vary according to the animal species involved and type of toxicity studies. Acute involves single or multiple exposures within 24 hrs. Sub-chronic toxicity duration involves a longer duration study which may vary from a weeks to month/s. Chronic toxicity investigations usually intend to study life-long alterations in structure or function of an organism.

Numbers of animals:

The numbers of animals to be selected and involved also vary accordingly. For example, if mice are to be used, the number should be more so as to cover all hematological parameters as required. [9] Satellite groups of animals may also be maintained appropriately.

In vivo studies also involve use of knock out mouse models such as one for liver fibrosis studies[10]Likewise variety of engineered mouse are used [8]

Liver Toxicity induction:

Liver toxicants are of different varieties and their mechanism of action / biotransformation also varies accordingly. As reviewed by Mueller et al the toxicant or the parent compound itself may induce the toxicity.SecondalyAny of toxicant's metabolite produced during its biotransformation may also induce the toxicity to the cells (2). However there is third possibility of induction of toxicity by toxicant as well as its metabolite (3)[7]

Chemical models to induce oxidative stress:[11]

Carbon tetrachlorideis the most widely used model to develop oxidative stress and liver toxicity in rats. It is uded both *in vivo* and *in vitro*. Mode of toxicity involves cytochrome pathways, mostly CYP2E1, CYP2B1, CYP2B2 and possibly CYP3A.

t-BHP: This model can be utilized as a pro-oxidant to achieve necrosis through induction of mitochondrial reactive oxygen formation.

Ethanol: Induction of CYP2E1 by ethanol is a central pathway by which ethanol generates oxidative stress.

Paracetamol: Paracetamol is primarily metabolized by sulfation and glucuronidation but with an increasing dose rate, these pathways become saturated and a greater proportion of the

drug is available for oxidation by microsomal cytochrome P-450 system, where NAPQI is the product of this pathway causing hepatic damage.

D-galactosamine (GAL): D galactosamine is mainly used for acute hepatic toxicity studies. It is capable of depleting the uridine pool of liver cells causing increased sensitization to cytokines.

Nicotine: Nearly 80% of the nicotine absorbed in the blood is metabolized by the liver, causing oxidative stress, however it is not recognized as a common experimental models for liver injuries and hence not well-established.

Liver fibrosis Models: To achieve fibrotic liver, three modes can be used *viz.* chemical, surgical or immunological. Carbon tetrachloride, thioacetamide, and dimethylnitrosamide are utilized achieve chemical induced liver fibrosis. Surgical method of bile duct ligation can lead to periportal hepatocyte death. Concanvalin A or schistomomamansoni are considered as immunological methods of inducing hepatic fibrosis. [12]

Biomarkers of hepatotoxicity studies.

Biomarkers of hepatotoxicity can be pathological, biochemical or in the current trends the omics markers are also studied.

Pathology :Hepatotoxicity is expressed by various pathological lesions viz. zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions, neoplasm, veno-occlusive

Biochemistry : Biochemical markers of hepatotoxicity are numerous and include, Aminotransferases (ALT and AST), Alkaline phosphatase, Y- Glutamyltransferase, Total UrobilinogenBile acids, Prothrombin time, Lactate dehydrogenase, Sorbitol dehydrogenase ,Glutamate dehydrogenase, Albumin, Total protein ,Serum F protein, Glutathione-S-transferase, ArginaseI, Malate dehydrogenase, Purine nucleoside phosphorylase, Paraoxonase 1, etc.

Omics markers : Potential omics markers of liver function are summarized by Xi Yang et al (2012).[13] Liver tissue can be studied for Interleukin-I, Annexin family of proteins, carbonic anhydrase III, Aflatoxin BI aldehyde reductase and 5-oxoprolin. Plasma can be utilized for cytokines, Interleukin-I, and possibly miRNA. Serum is source for Aflatoxin BI aldehyde reductase, glutathione S-transferase P-form, cytokeratin-I8, high mobility groupbox protein, malate dehydrogenase, purine nucleoside phosphorylase, paraoxanase-I, apolipoprotein E, bile acids, ophthalmic acid, Acylcarnitines and fatty acids. Amino acids can be studied for toxiconomics in liver tissue, urine and plasma while 5 oxoproline is studied in liver tissue, urine as well as in serum.

Concluding remarks: In lieu of tremendous work being carried on hepatotoxicity worldwide, the spectrum of work also varies from function tests to cell signaling. However every method and marker comes with its own advantages and dis-advantages. The researchers have a choice from huge number of models and markers according to their specific requirement.

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