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In Vitro Models to Study Hepatotoxicity

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ABSTRACT

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. Therefore, approaches to study hepatic toxicity in the early phase of drug discovery represent an important step towards rational drug development. A variety of in vitro liver models have been developed in the past years. Next to their use in drug development, they can also be applied to study environmental toxins and their hepatotoxicity. The 3 main approaches are ex vivo isolated and perfused organ models, precision-cut liver slices and cell culture models. Although the advantage of whole organ perfusions is based on the assessment of physiologic parameters such as bile production and morphologic parameters such as tissue histology, cell culture models can be efficiently used to assess cellular metabolism, cytotoxicity and genotoxicity. The advantage of precision-cut liver slices is based on the juxtaposition of cellular assays and tissue morphology. None of these models can be compared as they all focus on different fields of hepatoxicology. For the future, the ideal setup for testing the hepatic toxicity of a new compound could of primary studies in cell or slice cultures to assess cellular effects and secondary studies using ex vivo perfused organs to examine gross organ function parameters and histology.

Keywords. Liver; toxicology; liver slices; cell culture; perfusion.

INTRODUCTION

The development of new drugs consists of a variety of single steps leading from the discovery of pharmacological effects in cell and animal models to the assessment of toxicity and finally to the demonstration of efficacy and safety in humans (46, 90). Although the process that leads to the discovery of potent pharmacological effects has been reduced by rational drug designing in the past years, a variety of problems may occur in the preclinical and clinical development and lead to the failure of a compound. One of the most prominent factors that limits drug development is based on the compound's toxicity towards human health and therefore, using the field of investigational toxicology, different models have been established to assess this factor in an early stage.

Due to its anatomical position between the gastro-intestinal tract and the systemic circulation and its biochemical properties, the liver plays an important role in the metabolism of exogenous substances. A large amount of both nutrients and noxious substances reach the liver through intestinal uptake and portal vein flow. Next to major hepatic functions such as the uptake, storage, and release of peptides, amino acids, lipids, carbohydrates and vitamins, the liver is the principal organ in biotransformation processes of exogenous substances. Among them, numerous xenobiotics may have toxic effects. Apart from its role in the detoxication process of xenobiotics the liver may also convert substances into toxic products or be subject to the effects of compounds that are hepatotoxic, such as ethionine or galactosamine (95).

Hepatic drug metabolism occurs in the hepatocytes that represent with 80% of the total volume and 60% of the total cell number the predominant cell type found in the liver (44).

The potential of drug-based or environmental hepatotoxins to generate liver cell injury results from a complex interaction of cellular processes and is based on direct or indirect reactions of the toxins with basic hepatocyte constituents such as proteins, lipids, RNA, or DNA. The most frequent hepatic reaction towards toxic effects is hepatic steatosis but a variety of many other lesions are known (96). In general, the reactions leading to these organ injuries all involve specific sequences that can be analyzed at the molecular, cellular, or organ level. Whereas in vivo studies on hepatotoxicity are limited by animal welfare/ethical concerns and difficulties to distinguish primary and secondary toxic effects, in vitro liver preparations are increasingly used as they offer different approaches on all levels of investigational toxicology.

In Vitro Liver Models

There are 3 different major models to study the hepatotoxic effects: The most frequently used model is the liver cell culture model that can be applied to examine effects of

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TABLE 1.—Advantages and limitations of different in vitro liver models.

Model	Advantages	Limitations
Isolated cells	All species including humans Whole livers or biopsies as source Information on cellular toxicity Cryopreservation Several compounds at different concentrations	No bile measurement No cell-to-cell interaction No preserved anatomy
Liver slices	Lobular structure partly preserved All species including humans Whole livers or biopsies as source Information on cellular toxicity Several compounds at different concentrations	No bile measurement No cell-to-cell interaction No preserved anatomy
Isolated organs	Closest to in vivo conditions Anatomy preserved Bile flow preserved Hematodynamic parameters can be assessed	Short-term viability (2–4 h) Only a few compounds can be assessed with one organ No studies on human liver High number of animals used Complexity of the setup

drugs/toxins on isolated hepatocytes on the cellular level. In contrast, isolated perfused organs display an approach towards the assessment of organ physiology and morphology and represent the closest model to the in vivo situation. As a bridge between these two approaches, precision-cut liver slices can be used to examine cellular aspects of liver toxicology in a tissue-specific background.

Each of these approaches has a number of advantages and disadvantages and therefore based on the specific toxicological question, a model selection should be performed when investigating drug- or environmental substances-induced hepatic toxicity (Table 1).

Isolated Liver Cell Models

To date, cultured liver cells represent the most frequently used in vitro liver cell model. These models usually consist of isolated hepatocytes and have been established as valid in vitro toxicological models for many years (4). Next to rat and other rodent hepatocytes, even human-cultured hepatocytes have been established as models to study hepatic drug metabolism and genotoxic potential of substances (20, 21, 53). Also, the characteristics of human hepatocytes were compared to other rodent and primate models (14, 38, 100).

The methodology of liver cell cultures has been improved over the last years and currently, a variety of isolation, culture, and cryopreservation models have been established and evaluated (99). Shortly after the first description of hepatocyte isolation by collagenase- and hyaluronidase-dissociation (61, 62), new improvements were made by perfusing the livers in situ with the dissociating agents (12). Later, the protocol of the 2-step collagenase technique for rat hepatocytes has been established (84, 85), which was later modified (98) and works well for short-term cultures of hepatocytes of a variety of species including rat, monkey, pig, dog, rabbit, and human. For long-term models, a variety of approaches including addition of basement membrane (83, 88), culture in a collagen sandwich (32, 65), or coculture with epithelial cells (78) has been proposed.

To guarantee the survival of hepatocytes isolated from individual donors, cryopreservation or cold storage techniques can be applied that lead to an indefinite (23, 29) or 48-hour (54, 77) extension, respectively. However, the viability of stored cells is much lower than that of freshly isolated hepatocytes and dependent on factors such as initial cell integrity, ice crystal formation, and hypoxia during freezing and toxicity of cryopreservation substances.

In contrast to other liver cell types such as immortalized hepatoma cell lines, hepatocytes have the capacity for biotransformation, which is crucial for toxicological studies. However, they lose activity of specific systems such as cytochrome P450 if compared to in vivo levels. Therefore specific strategies using the addition of matrix materials or agents have been developed to maintain metabolizing activity (8, 9, 87). Studies addressing the metabolizing capacity of human, rat, dog, and monkey cultured hepatocytes revealed that for the model drug adinazolam, 24 hours after isolation the cells still generated metabolites similar to those formed in vivo; therefore, it was concluded that for at the least the first few days, cultured hepatocytes may be used to identify potential toxic metabolites (99).

Cultured hepatocytes have been used as applications in investigative toxicology for a variety of questions so far. In this respect, to investigate the mechanisms of hepatic toxicity, a combination of cell functional and cytotoxicity assay displays a useful approach. For cytotoxicity assays, propium iodide uptake or lactate dehydrogenase (LDH) release and for functional assays, levels of cytochrome P450 (13, 53) or structural alterations (27) may be measured on a cellular level. Based on these methodological aspects, a variety of studies used cultured hepatocytes to study liver toxicology (6, 7, 28, 93, 100, 101, 105).

A significant disadvantage of hepatocyte cultures is the absence of organ-specific cell-to-cell interactions. In this respect, liver hepatocytes show a marked heterogeneity along the porto-central axis regarding enzyme activity or subcellular architecture resulting in phenotypes that alter within different zones of the liver lobuli. This zonation also affects drug-metabolizing enzymes such as some cytochrome P450 isoenzymes, NADPH-cytochrome c reductase or UDP-glucuronyl transferase enzymes (44, 45). Although only a few drug-metabolizing enzymes have been reported in other hepatic cell types such as endothelial, Kupffer, biliary epithelial cells or fibroblasts (66–68, 76, 82), their direct or indirect role in drug-induced hepatotoxicity is well documented (33, 36, 60, 73).

Isolated Perfused Organs

In contrast to isolated hepatocyte models, the isolated perfused liver represents the closest in vitro model of the in vivo situation. Since the first use of isolated perfused livers for physiologic research (47) and for treatment of patients with hepatic coma or prior to transplantation (2, 3, 22, 34), a large number of studies have focused on the establishment of valid isolated organ models. Contrary to kidney perfusion models that were first established using a variety of small animal models such as the rat (42) or rabbit (79), the first liver perfusion setups were primarily based both on porcine organs (31) and small animal organs (1, 56). The major advantages of the isolated perfused livers are the preservation of the 3-dimensional organ structure with all its cell-to-cell interactions and the possibility of real-time bile collection and analysis. Furthermore, this model allows the study of

hemodynamic parameters if blood is used as a perfusate (24–26, 72).

So far, the rat model of isolated perfused liver has been used in a variety of studies for the investigation of drug- and chemical-induced hepatotoxicity. A variety of substances have been examined including aliphatic alcohols (91), thioacetamide (71), acetaminophen (17), methacrylate (11), cyclosporine (30), insecticides such as lindane or mirex (94, 104), phalloidin (16), solvents such as dimethylacetamide (74), metals such as mercury, cadmium, copper, vanadium, or aluminium (18, 92, 106), aromatic amines (5, 57), muscle relaxants such as atracurium (19), or antibiotic agents such as nitrofurantoin (86).

However, as small laboratory animal organ models such as the rat model have significant differences in organ size, function, and geometry compared to the human liver, isolated perfused porcine, canine or bovine livers display a better approach to simulate human in vivo conditions. A variety of different porcine liver perfusion models have been described so far (2, 31, 37, 55, 64). In all these studies, the proper assessment of liver viability is essential and it is generally agreed that the levels of oxygen consumption and bile acid production are reliable parameters.

Asanguineous perfusates were used in the majority of studies (2, 58, 59, 64) and only in a few studies, autologous blood was used (10, 63, 81). In contrast to blood-free perfusion setups, these studies allowed to assess hepatic parameters under physiological perfusion conditions with autologous blood. The blood pressure can be kept within physiological ranges in both hepatic artery and portal vein as compared to human parameters. In contrast, the continuously increasing levels of AST, ALT, and LDH were suggested to be a result of model-specific liver cell injury due to ischemic damage and hemolysis after reperfusion injury, an effect, which has to be taken into consideration in all ex vivo perfused organ models (37).

Altogether, the different models of isolated perfused livers proved to be very complex in keeping organ function within physiological ranges, and their functional integrity was never maintained over a prolonged period. Furthermore, the establishment of these models is very expensive and ethical concerns about animal welfare prevent a more widespread use. However, future studies using organs from abattoirs may resolve ethical and economic problems. Also, the integration of powerful morphological and molecular biological methods such as immunohistochemistry (52, 69), RT-PCR (51), northern blotting (50), mRNA in situ hybridization (40, 49), or uptake studies (48) could significantly improve the impact of perfused organs.

Precision Cut Liver Slices

In view of the limitations of cell culture models with missing cell-to-cell interactions and the complexity of isolated perfused organs, new methods using liver slices were established. Dating back to 1923, liver slices were first prepared by Otto Warburg (35). The liver-slice models using precision-cut slices that were established in past years (43, 75) retain tissue organization and cell-to-cell matrix interactions such as perfused organs. However, bile flow and functional parameters such as portal flow cannot be analyzed. Due to the development of new tissue-slicer models, the main methodological problems of the liver slices that are represented by the poor diffusion of oxygen and nutrients have been improved. Now, slices in dimensions of less than 250-μm thickness can be prepared with minimal tissue trauma (89). Recent studies demonstrated that the system can be used for periods of 2 to 3 days as a valid model to study hepatotoxicity and even human tissues can be used after surgical or needle biopsy removal (103).

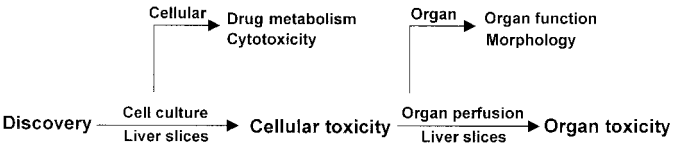

FIGURE 1.—In vitro liver toxicology models for drug discovery and development process.

Liver-slice models have been used in investigational pathology to assess a number of hepatotoxic effects and a variety of substances have been studied such as halogenated hydrocarbons (41), paracetamol (70), aflatoxin B1 (102), endotoxin (80), paraquat (97), cocaine (15), or metals such as zinc (107). Although the main advantages are represented by the preservation of lobular structures in contrast to cell cultures and the possible application of biochemical and molecular biological methods in contrast to organ perfusions, the main disadvantages are based on the short viability and the missing bile collection.

CONCLUSIONS

Over the last decades increasing costs of research coupled with animal welfare concerns have led to the development of new powerful in vitro models for the assessment of drug-induced hepatotoxicity. These models can also be used for the field of investigational pathology of environmental toxins. The 3 main approaches are represented by liver cell culture models, precision-cut liver slices, and ex vivo isolated and perfused organs. Each of these models has significant advantages with respect to specific scientific questions. Although the advantage of whole organ perfusions is based on the assessment of physiologic parameters such as bile production and morphologic parameters such as tissue histology, cell culture models can be efficiently used to assess cellular metabolism, cytotoxicity, and genotoxicity and lead to a reduction of laboratory animal use. The major future challenges will be to further improve the viability of each method, to establish a useful link between them (Figure 1) and to integrate new promising techniques such as laser-assisted cell harvesting (39).

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