Evaluation of a Liver Microfluidic Biochip to Predict *In Vivo* Clearances of Seven Drugs in Rats

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ABSTRACT: We investigated metabolic clearances of phenacetin, midazolam, propranolol, paracetamol, tolbutamide, caffeine, and dextromethorphan by primary rat hepatocytes cultivated in microfluidic biochips. The levels of mRNA of the HNF4α, PXR, AHR, CYP3A1, and CYP1A2 genes were enhanced in the biochip cultures when compared with postextraction levels. We measured a high and rapid adsorption on the biochip walls and inside the circuit for dextromethorphan and midazolam, a moderate adsorption for phenacetin and propranolol, and a low adsorption for caffeine, tolbutamide, and paracetamol. Drug biotransformations were demonstrated by the formations of specific metabolites such as paraxanthyne (caffeine), paracetamol (phenacetin), 1-OH midazolam (midazolam), paracetamol sulfate (paracetamol and phenacetin), and dextrorphan (dextromethorphan). We used a pharmacokinetic model to estimate the adsorption and *in vitro* intrinsic drug clearance values. We calculated *in vitro* intrinsic clearance values of 0.5, 3, 12.5, 83, 100, 160, and 900 μL/min per 10^6 cells for the tolbutamide, caffeine, paracetamol, dextromethorphan, phenacetin, midazolam, and propranolol, respectively. A second model describing the liver as a well-stirred compartment predicted *in vivo* hepatic clearances of 0.1, 13.8, 30, 44.1, 61, 72, 85, and 61 mL/min per kg of body mass for the tolbutamide, caffeine, paracetamol, midazolam, dextromethorphan, phenacetin, and propranolol, respectively. These values appeared consistent with previously reported data. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:706–718, 2014

Keywords: hepatic clearance; in vitro model; mathematical model; pharmacokinetics; microfluidic biochips; drug metabolism; rat hepatocytes

INTRODUCTION

Because of its major role in the biotransformation and detoxification of xenobiotics, the liver is considered to be one of the main determinants of the internal dosimetry of xenobiotics, in terms of the amount of compound available and residence time in the body. Many approaches have been developed to predict *in vivo* hepatic clearance using *in vitro* methodologies, such as suspensions of microsomes or hepatocytes[1,2] and monolayer cultures,[3] associated with mathematical models to extrapolate from *in vitro* to *in vivo* based on physiological clearance concepts.[4–7]

Numerous studies have been carried out to develop experimental protocols and mathematical models that provide the most reliable predictions for *in vivo* hepatic clearance or *in vivo* intrinsic clearance. For example, the use of hepatocytes is now widely accepted.[8,9] Hepatocytes effectively have the phase I and phase II metabolic activities in the liver and cell membranes in which drug transporters are functional. In this respect, hepatocytes are considered to be a more appropriate *in vitro* source for reflecting metabolic clearance in the liver than liver microsomes, which mainly represent microsomal phase I metabolic activity.[10,11] In addition, the presence of serum in the culture medium might lead to better prediction of *in vivo* clearance as it is believed to mimic *in vivo* protein binding.[12,13]

The impact of the *in vitro* device has also been investigated.[3,8,14] Currently, one of the most commonly used systems is the suspension of hepatocytes, which makes possible a rapid distribution of the drug to the metabolizing enzymes. However, the use of hepatocytes in suspension is limited (e.g., short incubation times) because they maintain viability for only a short time after isolation. As an alternative, Griffin and Houston[3] tested monolayer cultures of hepatocytes to prolong the maintenance of liver-specific functions. Even though the predictions in monolayer cultures were lower for high-turnover compounds compared with suspensions, they observed that better predictions were obtained in the case of a low-turnover compound (*S*-warfarin).

Recently, new *in vitro* systems have been developed based on miniaturized perfusion systems reproducing a miniaturized liver.[15,16] These systems make it possible to reproduce a culture environment similar to the *in vivo* situation, and aim to maintain the functionality of the cultured cells. Several studies presented as "proof of concept" have been performed to assess liver metabolism.[17–21] Qualitative examination of these systems was needed to find the configuration that would be most predictive of *in vivo* metabolism.

The aim of this study is to evaluate the performances of a hepatic biochip in predicting the hepatic clearance of seven

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therapeutic molecules (phenacetin, midazolam, propranolol, paracetamol, tolbutamide, caffeine, and dextromethorphan). This microsystem makes it possible to rearrange the cells in three dimensions. Previous studies showed a good adaptation of liver cells to the dynamic environment culture[16,22] and the maintenance of the expression of the enzymes related to drug metabolism. In this work, we develop a kinetic model to describe the fate of the drugs in the perfused system. Nonspecific binding in the system will be considered. The predictions of the *in vivo* intrinsic clearances and hepatic clearances will be compared with predictions obtained with literature data.

MATERIALS AND METHODS

Integrated Dynamic Cell Culture in Microsystems Bioreactor

Bioreactor principle

The entire culture set-up was called the IDCCM for "Integrated Dynamic Cell Culture in Microsystems." The concept and detail of the IDCCM platform and biochips are presented in detail in our previous work.[22,23] The IDCCM box is the result of the integration of 12 microfluidic biochips into a larger fluidic platform making parallelized cell cultures possible. The microfluidic biochips are made of polydimethylsiloxane using a molding process. They were connected at the bottom of the IDCCM platform by a simple series of "plugging" ports (Figs. 1a–1c). The IDCCM is a manufactured polycarbonate using the conventional format of a 24-well plate. Each microfluidic biochip was connected between two wells of the polycarbonate box (as illustrated in the diagram in Fig. 1a). Twelve wells were used as the entrance and 12 wells as the outlet reservoir leading to the parallelized culture of 12 biochips (Fig. 1c). A specific cover was designed for hermetic closure of the polycarbonate box to make continuous flow perfusion possible (Fig. 1d). The cover includes ports for fluid perfusion and sampling. The hermetic closure of the IDCCM box and the pressure inside the IDCCM box prevent any leakage or reservoir drain.

Primary Rat Hepatocyte Culture in IDCCM

The experiments were performed during a period of 48 h, which included three different phases: hepatocyte extraction (day 0, postextraction), the adhesion phase (day 1, 24 h of adhesion), and 24 h of perfusion phase (Fig. 1e). The biochips, IDCCM box, and perfusion circuit were sterilized by autoclaving the whole set-up. The biochips were then connected to the box under sterile conditions. Details of the optimization of the cell cultures and the rat hepatocyte cultures in an IDCCM box are presented in our previous works. Briefly, we investigated the hepatocytes response (via Reverse Transcription-quantitative Polymerase Chain Reaction , RTqPCR analysis of drug metabolism related genes, glucose consumption, albumin production, and

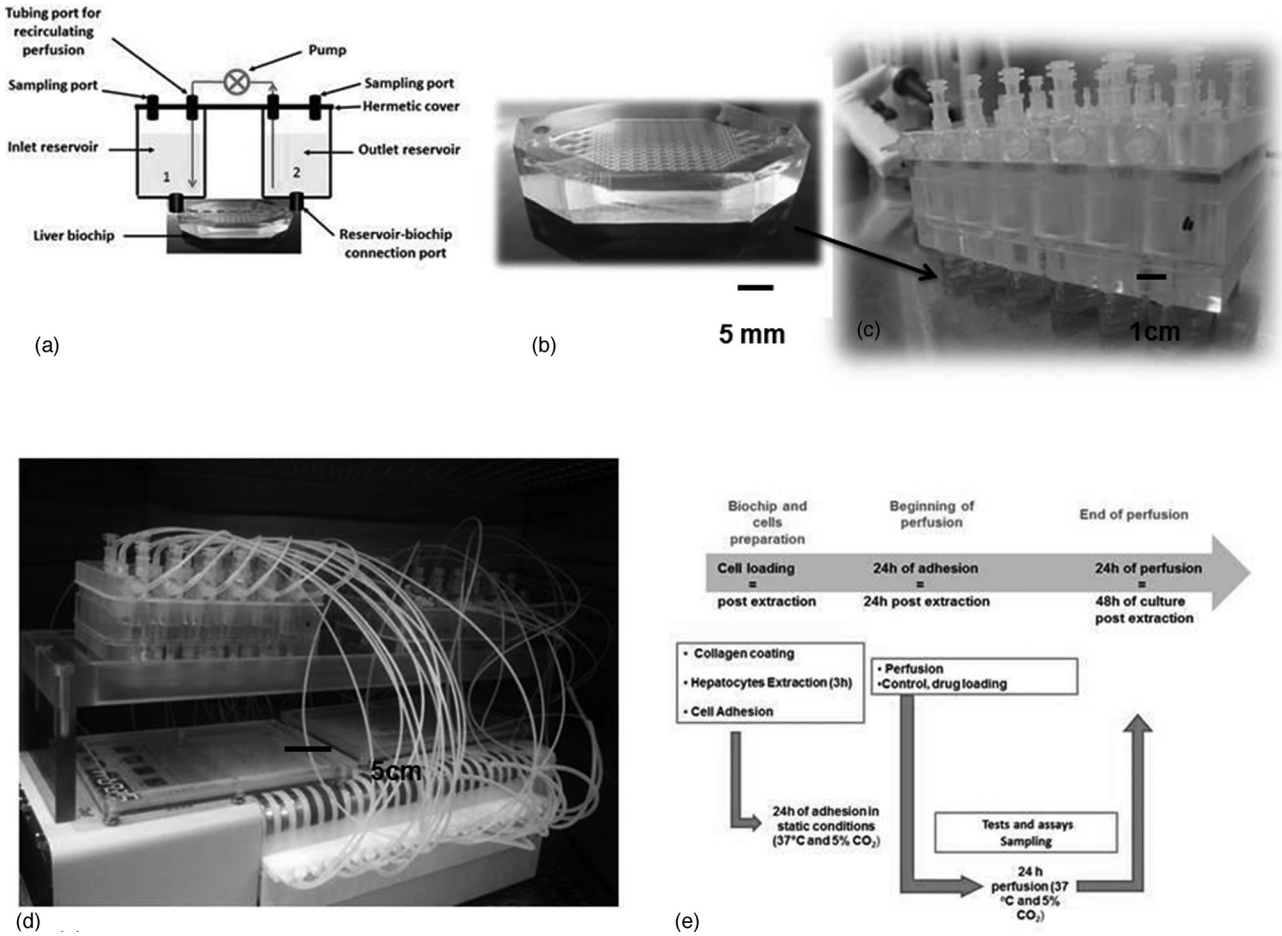

Figure 1. (a) Concept of the IDCCM biochip platform. (b) Microfluidic biochip. (c) IDCCM platform with ports and connected biochips. (d) IDCCM platform and biochip set-up on perilstatic pump in a CO_2 incubator. (e) Experimental set-up.