Available online at www.sciencedirect.com
ScienceDirect
Chemico-Biological Interactions 168 (2007) 16–29
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Human hepatocytes: Isolation, cryopreservation and applications in drug development

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Available online 9 January 2007

Abstract

The recent developments in the isolation, culturing, and cryopreservation of human hepatocytes, and the application of the cells in drug development are reviewed. Recent advances include the improvement of cryopreservation procedures to allow cell attachment, thereby extending the use of the cells to assays that requires prolong culturing such as enzyme induction studies. Applications of human hepatocytes in drug development include the evaluation of metabolic stability, metabolite profiling and identification, drug–drug interaction potential, and hepatotoxic potential. The use of intact human hepatocytes, because of the complete, undisrupted metabolic pathways and cofactors, allows the development of data more relevant to humans in vivo than tissue fractions such as human liver microsomes. Incorporation of key in vivo factors with the intact hepatocytes in vitro may help predictive human in vivo drug properties. For instance, evaluation of drug metabolism and drug–drug interactions with intact human hepatocytes in 100% human serum may eliminate the need to determine in vivo intracellular concentrations for the extrapolation of in vitro data to in vivo. Co-culturing of hepatocytes and nonhepatic primary cells from other organs in the integrated discrete multiple organ co-culture (IdMOC) may allow the evaluation of multiple organ interactions in drug metabolism and drug toxicity. In conclusion, human hepatocytes represent a critical experimental model for drug development, allowing early evaluation of human drug properties to guide the design and selection of drug candidates with a high probability of clinical success.

Keywords: Human hepatocytes; Hepatocyte isolation; Hepatocyte cryopreservation; Drug development; Drug metabolism; Drug–drug interactions; Drug toxicity; Toxicogenomics; IdMOC

1. Introduction

Primary cultures of hepatocytes represent an experimental tool that has been used extensively in biomedical research, both in academia as well as for commercial purposes such as drug development. The most exciting advances are the successful isolation, culturing, and cryopreservation of hepatocytes from human livers. Human hepatocytes are used routinely in drug development as an experimental model for the evaluation of key human-specific drug properties such as metabolic fate, drug–drug interactions, and drug toxicity. The applications range from the early screening the most appropriate new chemical entities for further development, to the determination of key drug properties for New Drug Applications (e.g. drug–drug interactions) to U.S. FDA.

Successful application of human hepatocytes in drug development requires a thorough understanding of the strengths and weaknesses of this valuable experimental system. This review is an effort to present a comprehensive review on the state-of-the-art of the isolation, cryopreservation, and applications of human hepatocytes

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doi:10.1016/j.cbi.2007.01.001

in drug development, with emphasis on the research performed in our laboratories in the past 20 years.

2. Human hepatocyte isolation

One of the major advances in human hepatocyte technology is the availability of human livers for research. In the United States, livers procured but not used for transplantation are allowed to be used in research. The major reasons that procured livers are not used for transplantation are as follows:

1. unavailability of a matched recipient;
2. physical damage to the liver;
3. pre-existing liver diseases;
4. breach of sterility during the procurement process;
5. high liver fat content;
6. inappropriate age (too young or too old);
7. inappropriate warm ischemic time;
8. inappropriate cold storage time.

Livers that are procured for transplantation are usually flushed extensively with a cold preservation solution, with the University of Wisconsin (UW) or histidine–tryptophan–ketoglutarate (HTK) solutions being the most common [1]. The rule of thumb is that viable hepatocytes can be obtained from human livers with up to 24 h of cold preservation. There are, however, cases that highly viable hepatocytes can be obtained from livers stored beyond this 24-h period.

Hepatocyte isolation from human livers is now universally performed with a "two-step" collagenase procedure developed by Berry and Friend [2]. Originally developed for the isolation of rat hepatocytes, this procedure has been modified by various laboratories for the isolation of hepatocytes from sevreal animal species, including human (e.g. [3,4]). The procedure involves the initial perfusion of the liver with a warm (37 °C) divalent ion-free, EGTA-containing, isotonic buffer (Step 1) to remove blood and to loosen cell–cell junctions, followed by perfusion with a warm, isotonic, collagenase solution (Step 2) to dissociate the liver parenchyma into single cells. In general, a higher amount of collagenase is required for the isolation of hepatocytes from human livers than that required for rat livers. As collagenase is a mixture of proteases, its composition can affect its effectiveness in the dissociation of the hepatocytes as well as its cytotoxicity. It is a common practice to evaluate multiple lots of collagenase to select the one lot yielding the highest number of viable hepatocytes from a liver. After digestion, the cells are harvested by low-speed centrifugation. A density gradient such as Percoll is commonly used to enrich for viable cells. The isolated cells can be used in suspension for experiments requiring a relatively short time duration (hours), plated on tissue culture surfaces pretreated with attachment substrates (e.g. collagen; Matrigel) for longer term studies, or cryopreserved for future use.

The method of isolation of human hepatocytes from a human liver is by no means optimized. Currently, a so-called "good" yield of human hepatocytes from a human liver is approximately 10–30 billion viable cells when a whole liver is perfused. Using an approximation of 1.5 kg as an average weight of a human liver, this leads to a yield of or approximately 7–20 million hepatocytes per gram of liver (e.g. [5,6]), which is considerably less that the total number of hepatocytes (approximately 300 billion) in the human liver. It is to be noted that the yield of human hepatocytes (in terms of number of hepatocytes per gram liver) is in general higher from smaller (e.g., 10 to 300 g) liver fragments then whole livers or lobes.

3. Cryopreservation

Hepatocytes, especially human hepatocytes, are now routinely used after they are cryopreserved [7,8]. The general procedures for hepatocyte cryopreservation have not deviated extensively from the original procedures [9]. Via the use of equipments to control freezing rates (e.g. programmable control-rate freezer) and appropriate cryopreservation agents (e.g. dimethyl sulfoxide), hepatocytes now can be stored in liquid nitrogen (lower than −150 °C) for an extensively time period (years) with the retention of high viability and drug metabolizing enzyme activity [7]. The most recent advancement of human hepatocyte cryopreservation is the ability of the thawed hepatocytes to be plated as monolayer cultures ("plateable" hepatocytes) [10,11]. In our laboratory, we routinely prepare cryopreserved human hepatocytes with most of the lots having post-thaw viability of >90% (Table 1) and with approximately half of the lots yielding over 50% confluent monolayer cultures when plated onto collagen-coated plates (Table 1; Fig. 1). In our laboratory, we believe that the key to successful cryopreservation is to ensure that the hepatocytes are isolated from the human liver with minimal damages to the plasma membrane.

It is important to fully understand the properties of cryopreserved human hepatocytes as compared to freshly isolated cells. The following are general conclusions on the drug metabolizing enzyme activities of cryopreserved human hepatocytes:

1. *P450 isoform activities*: Since the first publication showing similar P450 isoform activities between