Toxicology in Vitro 42 (2017) 200–213

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Maintenance of high quality rat precision cut liver slices during culture to study hepatotoxic responses: Acetaminophen as a model compound

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ARTICLE INFO

Keywords:
Precision cut liver slices
Hepatotoxicity
Acetaminophen
Culture conditions
Culture setup

ABSTRACT

Precision cut liver slices (PCLiS) represent a promising tool in reflecting hepatotoxic responses. However, the culture of PCLiS varies considerably between laboratories, which can affect the performance of the liver slices and thus the experimental outcome. In this study, we describe an easily accessible culture method, which ensures optimal slice viability and functionality, in order to set the basis for reproducible and comparable PCLiS studies. The quality of the incubated rat PCLiS was assessed during a 24 h culture period using ten readouts, which covered viability (lactate dehydrogenase-, aspartate transaminase- and glutamate dehydrogenase-leakage, ATP content) and functionality parameters (urea, albumin production) as well as histomorphology and other descriptive characteristics (protein content, wet weight, slice thickness). The present culture method resulted in high quality liver slices for 24 h. Finally, PCLiS were exposed to increasing concentrations of acetaminophen to assess the suitability of the model for the detection of hepatotoxic responses. Six out of ten readouts revealed a toxic effect and showed an excellent mutual correlation. ATP, albumin and histomorphology measurements were identified as the most sensitive readouts. In conclusion, our results indicate that rat PCLiS are a valuable liver model for hepatotoxicity studies, particularly if they are cultured under optimal standardized conditions.

1. Introduction

Drug-induced liver injury (DILI), reflecting both, the intrinsic as well as the severe idiosyncratic form, is a multifaceted form of organ damage, which still represents a major pitfall in the drug development process and for the safety of newly marketed drugs (Fung et al., 2001; Lee, 2003; Uetrecht, 2008; Roth & Ganey, 2010; Watkins, 2011; Goldring et al., 2015). The intercellular communication of parenchymal hepatocytes with non-parenchymal cells such as liver resident macrophages (Roberts et al., 2007; Adams et al., 2010) as well as zone-specific damage (Kleiner et al., 2014) play an important role in DILI. Liver models with the relevant cell types and structural features of the in vivo tissue would thus improve the ability to predict hepatotoxicity in the early pre-clinical screening process of drug candidates and thereby enhance their safety. Currently, widespread used in vitro models for the prediction of DILI often lack the three-dimensional architecture and multicellular complexity of the target organ and therefore cannot mimic crucial tissue dynamics in vitro (Brandon et al., 2003). Precision cut liver slices (PCLiS), a liver model that is used by a growing number of researchers to study a number of open questions in the areas of toxicology, pharmacology and the metabolism of xenobiotics (Lerche-Langrand & Toutain, 2000; Lake & Price, 2013; Olinga & Schuppan, 2013), retain the original liver tissue architecture with all naturally occurring cell types and offer a great variety of possible readouts (Lerche-Langrand & Toutain, 2000; Vickers & Fisher, 2005; de Graaf et al., 2010). Importantly, in addition to the frequently used biochemical methods in in vitro/ex vivo models, clinically relevant biomarkers and histomorphological techniques can be applied to the PCLiS model. This test system, which mirrors to a great extent the in vivo situation in the liver, might help in finding a more reliable liver

Abbreviations: AST, aspartate transaminase; BSA, bovine serum albumin; C_{max}, maximal plasma concentration; DILI, drug-induced liver injury; EC50, half maximal effective concentration; GLDH, glutamate dehydrogenase; KH, Krebs-Henseleit; LDH, lactate dehydrogenase; NL, normal liters; PCLiS, precision cut liver slices; SD, standard deviation; SER, smooth endoplasmic reticulum

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http://dx.doi.org/10.1016/j.tiv.2017.05.001
Received 9 December 2016; Received in revised form 13 March 2017; Accepted 1 May 2017
Available online 02 May 2017

model to predict human DILI. However, this promising liver model can only be as good as its performance and stability and the reproducibility of the results obtained with it. These fundamental quality aspects of the system strongly depend on the use of high quality tissue and, just as important, on a culture setup, system and protocol that together ensure optimal culture conditions in order to maintain the slice viability and functionality and to accurately reproduce an in vivo outcome (Vickers & Fisher, 2005; de Graaf et al., 2010). Although a great effort has been made to optimize the PCLiS technique, there is no consensus among the laboratories regarding the culture setup, system and protocol as well as the longest possible incubation period that will support an optimal tissue performance. While the preparation of the slices is very similar between laboratories and everyone agrees that PCLiS can be better maintained in dynamic cultures (Fisher et al., 1995; Olinga et al., 1997a, 1997b; de Graaf et al., 2007, 2010) and in an atmosphere with an enhanced oxygen concentration (Drobner et al., 2000; Evdokimova et al., 2002; Szalowska et al., 2013), there are great differences in the used culture setup (incubator, gas supply) and system (culture container), the applied oxygen concentration in the culture atmosphere, the culture medium including the added supplements, the pre-incubation period, the total culture period and whether a perfusion of the liver is performed before its removal or not (compare Supplementary Table 1). Based on this high number of possible variations, it becomes obvious that the extremely different management of PCLiS cultures in each particular laboratory can affect the performance of the liver slices in a different way and to a different extent, thereby strongly influencing the experimental outcome and preventing the routine use of this valuable liver model. Both may lead to results that are neither representative nor comparable. Many working groups are aware of this fact and make great effort to prove the functionality and viability of their cultured PCLiS before they start their actual study (Karim et al., 2013; Koch et al., 2014; Westra et al., 2016). To prevent the need of this additional expense and to increase the reproducibility of results in future PCLiS studies, our aim was to demonstrate that as long as the PCLiS culture is performed under optimal conditions representative and comparable results can be obtained. A standardized culture method ensuring optimal culture conditions would furthermore allow similar liver slice quality and outcomes regarding model hepatotoxicants at different laboratories enhancing the comparability of PCLiS studies. To this end we established a simple and easily accessible culture setup, system and protocol for the optimal maintenance of PCLiS during culture and characterized extensively the incubated liver slices regarding their quality. For a better comparability, our study was performed with liver tissue from the most studied animal species, the rat, and the most accepted and used culture parameters based on a comprehensive literature search. The characterization was based on a number of different readouts including viability (lactate dehydrogenase [LDH]-, aspartate transaminase [AST]- and glutamate dehydrogenase [GLDH]-leakage, ATP content) and functionality parameters (urea and albumin secretion) as well as histomorphology and other descriptive characteristics (protein content, wet weight and thickness of the slices). After verifying the performance of the rat PCLiS during a 24 h culture period, we exposed the PCLiS to increasing concentrations of the model compound acetaminophen (APAP) to assess the significance of the ten readouts in the evaluation of hepatotoxic responses. In this context, we furthermore aimed to identify whether our cultured PCLiS provide a robust basis for the generation of reproducible and comparable responses. By presenting a well-described and -characterized culture setup, system and protocol, which ensure an optimal tissue quality during a 24 h culture, as well as meaningful readouts, we contribute to push forward the standardization of the PCLiS technique and to improve the reproducibility of PCLiS studies.

2. Materials & methods

2.1. Animals

Male Wistar rats (Crl:WI(Han); 220–240 g; 7 weeks old) were obtained from Charles River (Sulzfeld, Germany). Three animals per Makrolon Type III cage were housed for one week under standard environmental conditions (20 ± 2 °C, 55 ± 15% relative humidity and 12 h dark/light cycles) for acclimatization before sacrification. They were fed with the standard diet ssniff V 1534 (ssniff-Spezialdiäten GmbH, Soest, Germany) and had access to water ad libitum. All experiments were conducted in accordance with the German Animal Protection Act.

2.2. Excision of rat liver

The non-fasted rats were sacrificed under Narcoren®-induced deep anesthesia (160 mg pentobarbital/kg BW; Merial GmbH, Hallbergmoos, Germany) by exsanguination via an incision of the vena cava caudalis. Thereafter, the liver was removed and immediately placed in ice-cold, sterile and oxygenated Krebs–Henseleit (KH-) buffer (pH 7.4, with 11 mM glucose) (Sigma-Aldrich GmbH, Taufkirchen, Germany) supplemented with 25 mM $NaHCO_3$ (Sigma-Aldrich) and 2.5 mM $CaCl_2 \cdot H_2O$ (Sigma-Aldrich), to prevent warm ischemia. Oxygenation of the buffer was achieved by saturation with carbogen (Carbogen®LAB (95% O_2, 5% CO_2); Linde Gas GmbH, Stadl-Paura, Germany). For this purpose, the ice-cold buffer was gassed with 3 normal liters (NL) carbogen/min for 30 min. The slice preparation started about 10 min after liver removal.

2.3. Preparation of PCLiS

With the help of a biopsy punch (pfm medical AG, Cologne, Germany), tissue cylinders (Ø 8 mm) were punched out of the whole liver without distinction of the lobes and collected in ice-cold oxygenated KH-buffer on melting ice until slicing. Cylindrical liver cores were placed in a Krumdieck tissue slicer MD 6000 (Alabama Research and Development, AL, USA) filled with ice-cold oxygenated KH-buffer and pushed down with a weight (5.68 g). Tissue slices of 249 ± 33 µm thickness and a wet weight per three pooled slices of 58 ± 4 mg (approx. 19 ± 1 mg per slice) were cut. The slicing buffer and blade were replaced at least three times during the slicing process. The freshly cut slices were collected in ice-cold oxygenated KH-buffer on melting ice until culture (about 45 min cold ischemia time). Slices were inspected and those with processing-related defects were rejected. The selected slices for the culture were randomly assigned to 25 mL flasks.

2.4. Culture setup and incubation protocol for PCLiS

The culture setup was composed of four culture boxes (dimensions: approx. 11 × 11 × 21 cm, width × high × depth), which were located in an incubation shaking cabinet (Certomat® CT Plus; Sartorius Stedim Systems GmbH, Göttingen, Germany) in an atmosphere of 37 °C and 90% humidity (Fig. 1A). The boxes were gassed with humidified carbogen, which was enabled by intercalating a gas washing bottle (filled with ~125 mL sterile aqua dest.) in the incubator and connected in series with plug-in couplings. Thereby, it was possible to separate the boxes from one another without losing the accumulated gas within the boxes. Eight 25 mL flasks containing the culture medium and the slices were placed into each box and shaken gently (80 times per minute) for an optimal medium rotation and to limit the mechanical stress for the slices. The gassing regime included a flooding of the boxes with 4 NL carbogen/min for 10 min; thereafter, the gas flow was reduced to 0.8 NL/min during the rest of the culture period in order to compensate for any potential gas loss. Each time that the boxes were opened to handle the slices, for example for a medium exchange, the flooding