Bioscience Solutions

Poster #1350

A Novel *in vitro* Liver Cell Culture Flow System Allowing Long-term Metabolism and Hepatotoxicity Studies

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Introduction

Hepatotoxicity is a concern when developing new small molecule pharmaceutical compounds. One of the major reasons for drug withdrawal from market is hepatotoxicity[1] that does not arise immediately, but requires patients to have prolonged and repeated exposure to drugs. Animal models can be suitable for the detection of hepatotoxicity including long-term and repeat dosing studies to replicate this long-term phenomenon. However, animal models often do not accurately predict toxicity in humans due to species differences including differences in compound metabolism. While there is increased interest in using *in vitro* primary human hepatocytes (PHH) to predict liver toxicity in humans, PHH cultures undergo rapid de-differentiation in standard culture systems, thus limiting their utility in development of long-term repeat dosing models *in vitro*.

Objective

Here we present an alternative *in vitro* model that is based on the long-term culture of PHH in the Quasi Vivo® QV900 flow system. The QV900 system consists of six chambers that can be connected in flexible configurations. A peristaltic pump generates a cell culture medium flow that mimics blood flow and ensures a steady supply of nutrients, growth factors and oxygen as well as removal of waste products and continuous dosing with test compounds.

Material and Methods

Cells and Media: Cryopreserved Human Hepatocytes, Plateable, Induction Qualified (Lonza) were cultured according to the supplier's instructions[2]. Briefly, 400,000 cells were plated per one 12 mm collagen coated coverslips (Neuvitro). 4-6 hours post-seeding, Matrigel™ (Corning) was added in Hepatocyte Culture Medium (HCM™). 24 hours after seeding, coverslips were transferred to Quasi Vivo® QV900 trays (QV 900, Kirkstall, Lonza) or fresh 24-well plates, respectively. Medium was renewed daily (standard sandwich culture) or twice a week (QV900). Flow rate in the QV900 system was set to ~130 µL / min. For analysis of cell viability and albumin production, coverslips were transferred into fresh 24-wells plates for 1.5 hours to ensure optimal comparability of results. Viability / metabolic capacity of the culture was assessed via CellTiter-Blue® Cell Viability Assay (Promega) according to manufacturer's instructions. Albumin content in the supernatant was quantified with the Human Albumin ELISA Kit (Bethyl) according to manufacturer's instructions. CYP3A4 activity was assessed via the P450-Glo™ CYP3A4 Assay (Promega) using Luciferin-IPA as a substrate according to manufacturer's instructions. For measurement of metabolic activity, PHH were incubated with 200 µM testosterone, 250 µM bupropion and 100 µM phenacetin for 15 min or 8 µM midazolam for 10 min. Formation of 6β-hydroxy-testosterone, OH-bupropion, acetaminophen and OH-midazolam, was evaluated, as analyzed by LC/MS/MS (Biotranex). For measuring the inducibility of CYP3A4: PHH were incubated with 10 µM rifampicin or DMSO control for 72 hours before midazolam metabolite analysis or measuring CYP3A4 activity via P450-Glo™ CYP3A4 Assay (Promega).

Hepatocyte Viability / Metabolic Capacity

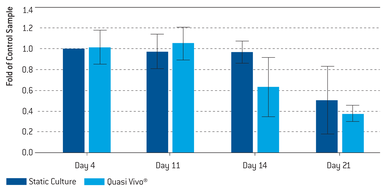


Figure 1: Viability – an indirect measure of metabolic capacity and cell number – of primary human hepatocytes is comparable in static and flow culture.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period of time in either static conditions or in QV900 flow culture. Viability was assessed with the CellTiter-Blue® Cell Viability Assay (Promega). Data of three independent experiments is shown, n ≥ 3. Error bars indicate standard deviation The control sample (day 4 standard culture) was set to 1 in every experiment for normalization.

Albumin Production

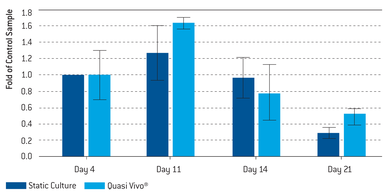


Figure 2: Albumin secretion of primary human hepatocytes is comparable in static and flow culture.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period in either static conditions or in QV900 flow culture. Albumin content in the supernatant was quantified with the Human Albumin ELISA Kit (Bethyl). Data of three independent experiments is shown, n ≥ 3. Error bars indicate standard deviation. The control sample (day 4 standard culture) was set to 1 in every experiment for normalization.

Morphology of Primary Human Hepatocytes

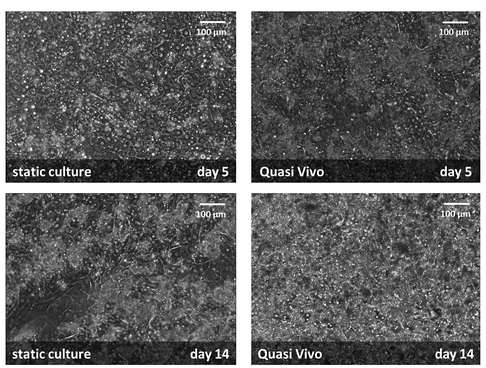


Figure 3: The morphology of primary human hepatocytes is comparable in static and flow culture.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period in either static conditions or in QV900 flow culture.

Basal CYP3A4 Activity

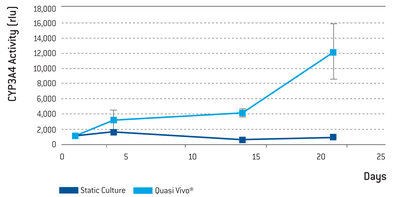


Figure 4: Increased basal CYP3A4 activity is observed in primary human hepatocytes cultured in Quasi Vivo™ QV900 flow culture.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period in either static conditions or in QV900 flow culture. CYP3A4 activity was assessed with the P450-Glo™ CYP3A4 Assay (Promega) using Luciferin-IPA as a substrate. Results of one typical experiment are shown, n = 3. Error bar indicate standard deviation.

CYP3A4 Activity: Testosterone Metabolism

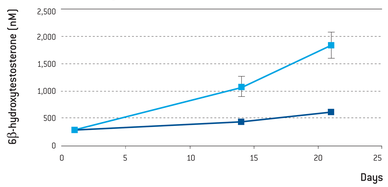
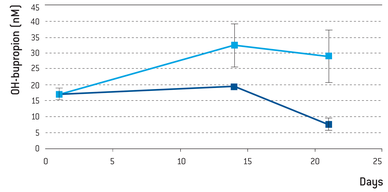
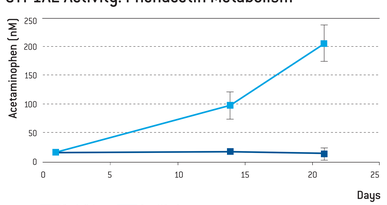


Figure 5: The basal activity of CYP3A4, CYP2B6 and CYP1A2 is increased in primary human hepatocytes cultured in QV900 flow culture.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period in either static conditions or in QV900 flow culture. (A) For measurement of CYP3A4 activity cells were incubated with 200 µM testosterone for 15 minutes and the formation of 6β-hydroxytestosterone was evaluated. (B) For measurement of CYP2B6 activity PHH were incubated with 250 µM bupropion for 15 minutes and the formation of OH-bupropion was evaluated. (C) For measurement of CYP1A2 activity PHH were incubated with 100 µM phenacetin for 15 minutes and the formation of acetaminophen was evaluated. All culture supernatants were analyzed by LC/MS/MS. Results of one typical experiment are shown, n = 3. Error bars indicate standard deviation.

CYP2B6 Activity: Bupropion Metabolism



CYP1A2 Activity: Phenacetin Metabolism



CYP3A4 Induction

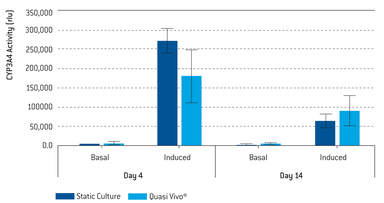


Figure 6: Primary human hepatocytes cultured in both static and flow conditions are responsive to Cyp3A4 induction. An 18- to 104-fold induction was observed in all samples.
PHH in collagen/Matrigel™ sandwich culture were cultured for the indicated period of time in either static conditions or in QV900 flow culture. 72 hours prior analysis, cells were stimulated with 10 µM rifampicin or DMSO control before measuring CYP3A4 activity with the luminescent P450-Glo™ CYP3A4 Assay (Promega). Results of one typical experiment are shown, n = 3. Error bars indicate standard deviation.

Results

When culturing PHH in the Quasi Vivo® QV900 system, we observed good cell viability (Figure 1) and sustained albumin production (Figure 2) for 14 days in culture. Even on day 21 albumin secretion was detectable. Healthy hepatocyte morphology was well maintained (Figure 3). In comparison to non-perfused cultures the basal activity of the drug metabolism enzymes CYP3A4, CYP1A2 and CYP2B6 was increased (Figure 4 and 5), e.g. by up to 10-fold for CYP1A2, as measured by the conversion of phenacetin to acetaminophen on day 14 and 21 of the culture. After 72 h of rifampicin treatment, induced CYP3A4 activity was observed perfused and non-perfused cultures, as assessed by luciferase assay (Figure 6) and analysis of hydroxymidazolam formation (data not shown). Furthermore, the Quasi Vivo™ QV900 system enabled the culture of PHH with two media changes per week only. In contrast, in non-perfused conditions, culture medium has to be changed on a daily basis. Therefore the QV900 systems facilitates the build-up of potential toxic metabolites that would be removed when media is refreshed daily.

Conclusion

In summary, we present a cell culture flow system that facilitates the long-term culture of primary human hepatocytes and improves longer-term stability of drug metabolism enzymes *in vitro*. Therefore, the presented system can serve as an advanced human *in vitro* liver model particularly suitable for long-term repeat dosing liver toxicity studies.

Literature

1. La Rochelle P. Analysis of the Drugs Withdrawn from the US Market from 1976 to 2010 for Safety Reasons. Pharm Med 2016; 30:277–289
2. Lonza. Suspension and Plateable Cryopreserved Hepatocytes – Technical Information & Instructions. Document # BR-CryoHep 05/17 Available: URL bio.lonza.com/go/literature/5334

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