HUMAN HepaRG™ CELLS, A USEFUL IN VITRO MODEL FOR CELL-BASED CHOLESTASIS ASSAYS

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Introduction

HepaRG ™ is a bipotent, human hepatoma line with a genetic profile that is in many ways similar to primary human hepatocytes. HepaRG™ influx and efflux transporters were correctly localized to canalicular (BSEP, MRP2, MDR1, MDR3) or baso-lateral (NTCP, MRP3) membrane domains and were functional (1).

Interestingly, cell imaging showed higher bile canaliculi contraction/ relaxation activity in HepaRG-hepatocytes. Total bile acids production by HepaRG-hepatocytes showed high inter-assay reproducibility and was in the same range as in primary human hepatocyte cultures with 316-320 pmoles/10^6 hepatocytes/day (2).

Altogether, our results bring new insights in mechanisms involved in bile acids accumulation and excretion in human Hepatocytes and suggest that HepaRG™ cells represent a suitable model for studying hepatobiliary transporters and drug induced cholestasis.

Material and methods

Cell cultures

HepaRG™ cells were seeded at a density of 2.6x10^4 cells/cm2 in Williams E medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin,100 mg/mL strep tomycin, 5 mg/mL insulin, 2mM glutamine, and 50 mM hydrocortisone hemisuccinate.

After 2 weeks, HepaRG™ cells were shifted to the same medium supplemented with 2% dimethyl sulfoxide for a further 2 weeks in order to obtain confluent differentiated cultures with maximum hepatocyte functional activities.

At this time, these cultures contained hepatocyte-like and progenitors/ primitive biliary cells.

Carboxy dichlorofluorescein excretion

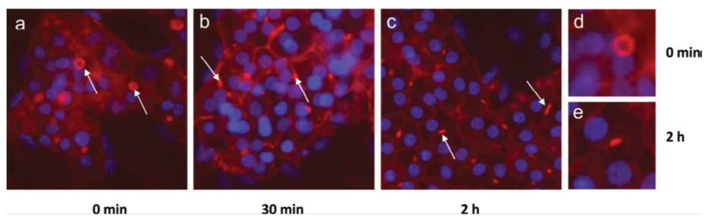
After 2h exposure with molecules known to be cholestatic ie Chlorpromazine (CPZ), Cyclosprine A (CsA) or Fasudil in serum-free medium, cells were incubated for 20 min at 37°C with 3 µM 5(6)-carboxy-2 ,7 -dichlorofluorescein diacetate (CDFDA), which is hydrolyzed by intracellular esterases to 5(6)-carboxy-2',7'-dichlorofluorescein (CDF), a substrate of MRP2 transporter.

Neosynthesis of bile acid measurement

The supernatant was collected and extracted using a SPE cartridge. BA content was measured using HPLC–MS/MS.

Results

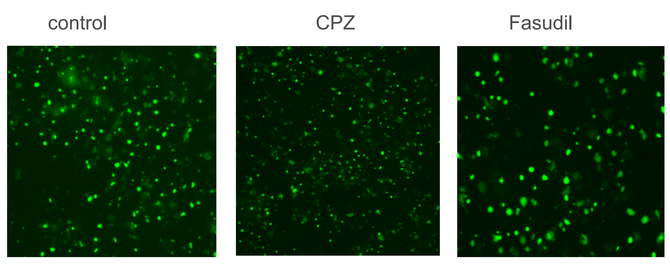
Bile canaliculi constriction induced by CPZ



Cells were treated with 50 µM CPZ for 30 minutes or 2 hours. F-actin was localized by using phalloidin-fluoprobe. Nuclei were stained in blue (Hoechst).

F-actin shows a predominant pericanalicular distribution in untreated cells and a less intense staining around the canalicular region in CPZ-treated cells. Although untreated cells show round shaped canaliculi, CPZ-treated cells exhibit retracted bile canaliculi (x20 magnification [a-c]). Arrows indicate bile canaliculi. Details of one canaliculus of untreated cells (d) and 2-hour CPZ-treated cells (e) are shown. Imaging quantification was done by using cellomics software .

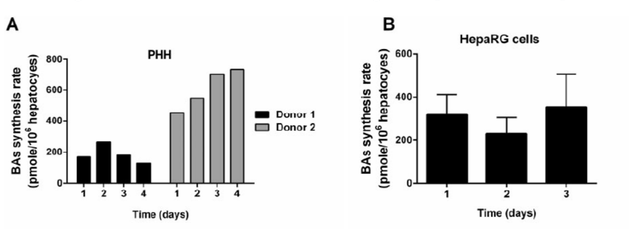
Bile canaliculi constriction or dilatation induced by cholestatic drugs



HepaRG-Hepatocytes were treated without or with 50 µM CPZ, or with 50µM Fasudil for 2 hours. CPZ treated cells exhibited canalicular constriction whereas treatment with Fasudil Induced dilatation of bile canaliculi.

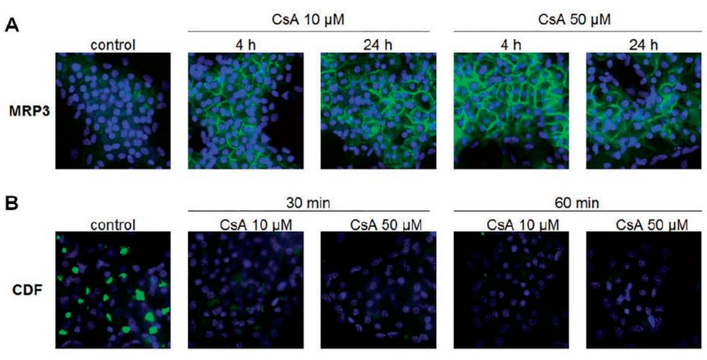
Canalicular size was evaluated by CDFDA accumulation (green color).

Neosynthesis of bile acid by HepaRG-hepatocytes



Culture in serum free medium with daily renewal.
Comparative levels are produced by HepaRG- and primary human hepatocytes (PHH) with high iner-assay reproducibility for HepaRG cells.

Exposure to CsA induced accumulation of MRP3 and inhibited MRP2 transporter activity in HepaRG-hepatocytes .



Representative immunofluorescence images of MRP3 basolateral localization in control and 10 and 50 µM CsA-treated HepaRG-hepatocytes, for 4 and 24 hours, and biliary MRP2 transporter activity using CDFDA substrate as fluorescent dye.

Discussion and conclusion

Drug-induced intra-hepatic cholestasis is characterized by intra-cellular hepatic accumulation of endogenous BAs which can cause toxicity.

- **HepaRG-hepatocytes mimi the biliary function of human hepatocytes:**
  - Neo-synthesis of bile acids
  - Dynamic of excretion at the biliary pole

- **HepaRG-hepatocytes respond to cholestatic drugs:**
  - Alteration of transporters activity
  - Change of the bile canaliculi size (dilatation or constriction)
  - Modification of the efflux dynamic

Altogether they highlight new insights in mechanisms implicated in disruption of BA secretion and evidence new potential predictive biomarkers of drug-induced cholestasis using HepaRG™.

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1-P.Bachour-El Azzi et al Comparative Localization and Functional Activity of the Main Hepatobiliary Transporters in HepaRG Cells and Primary Human HepatocytesTox Sci 2015, 145, 157-168
2-A.Sharanek et al , Cellular accumulation and toxic effects of bile acids in CsA-treated HepaRG hepatocytes. Toxicol Sci 2015
3-B L.Woolbright et al. Bile acid-induced toxicity in HepaRG cells recapitulates the response in primary human hepatocytes.Basic Clin Pharmacol Toxicol 2015
4-S.Antherieu et al, Oxidative stress plays a major role in chlorpromazine-induced cholestasis in human HepaRG. Hepatology 2013.

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