Phase 1 and phase 2 drug metabolism and bile acid production of HepaRG cells in a bioartificial liver in absence of dimethyl sulfoxide


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Abbreviations:
BAL, bioartificial liver; BSEP, bile salt export pump; CA, cholic acid; CYP, cytochrome P450; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; g-CA glycine-conjugated cholic acid; g-CDCA, glycine-conjugated chenodeoxycholic acid; HPLC, high performance liquid chromatography; MRP2, multidrug resistance-associated protein 2; NI, not identified; PBS, phosphate-buffered saline; PHHs, primary human hepatocytes; RT-PCR, Reverse transcription-polymerase chain reaction; t-CA, taurine-conjugated cholic acid; t-CDCA, taurine-conjugated chenodeoxycholic acid; UGT, UDP-glucuronosyltransferases; UPLC, ultra performance liquid chromatography; 6β-OHT, 6β-hydroxytestosterone
Abstract
The human liver cell line HepaRG has been recognized as a promising source for in vitro testing of metabolism and toxicity of compounds. However, currently the hepatic differentiation of these cells relies on exposure to dimethysulfoxide (DMSO), which, as a side-effect, has a cytotoxic effect and represses an all-round hepatic functionality. The AMC-bioartificial liver (AMC-BAL) is a 3D bioreactor that has previously been shown to upregulate various liver functions of cultured cells. We therefore cultured HepaRG cells in the AMC-BAL without DMSO and characterized the drug metabolism. Within 14 days of culture, the HepaRG-AMC-BALs contained highly polarized viable liver-like tissue with heterogeneous expression of cytochrome P450 (CYP) 3A4. We found a substantial metabolism of the tested substrates, ranging from 26% (UDP-glucuronosyltransferase 1A1), 47% (CYP3A4) to 240% (CYP2C9) of primary human hepatocytes. The CYP3A4 activity could be induced 2-fold by rifampicin, while CYP2C9 activity remained equally high. The HepaRG-AMC-BAL secreted bile acids at 43% the rate of primary human hepatocytes and demonstrated hydroxylation, conjugation, and transport of bile salts. Concluding, culturing HepaRG cells in the AMC-BAL yields substantial phase 1 and phase 2 drug metabolism, while maintaining high viability, rendering DMSO addition superfluous for the promotion of drug metabolism. Therefore, AMC-BAL culturing makes the HepaRG cells more suitable for testing metabolism and toxicity of drugs.
Introduction

The demand for safety testing of newly developed drugs rises due to stricter regulatory demands for market approval, as well as the increasing number of compounds to be tested. A large share of tests target the metabolism and toxicity of compounds in the liver (Stirnimann et al., 2010), as the liver is the primary site for drug metabolism and hepatocellular and/or cholestatic liver injury is a major cause of attrition in (pre-)clinical drug development.

Hepatic drug metabolism entails a myriad of chemical reactions that are also involved in the metabolism of endobiotics, such as bile acids and bilirubin. Two phases are recognized: phase 1, the basic alteration of structures predominantly catalyzed by cytochrome P450 (CYP) enzymes, and phase 2, the conjugation of a hydrophilic moiety to the drug by transferases, such as UDP-glucuronosyltransferases (UGT) and sulfotransferases. In addition, transport processes are relevant, both for uptake and excretion into either bile or back into the circulation.

The HepaRG cell line is recognized as a suitable resource for testing hepatic metabolism and toxicity of compounds (Andersson et al., 2012). The HepaRG cell line is a liver progenitor cell line that forms hepatocyte-like clusters surrounded by biliary epithelial-like cells within 28 days (Cerec et al., 2007; Gripon et al., 2002). Drug metabolism is upregulated when the culture is exposed to 2% dimethylsulfoxide (DMSO) during the last 14 days. DMSO-treated HepaRG cultures functionally express hepatic drug transporters (Le Vee et al., 2006), show phase 1 metabolic activity, with unique high CYP3A4 activity, expressed by the hepatocyte clusters (Cerec et al., 2007) and high transcript levels of phase 2 enzymes (Aninat et al., 2006). Moreover, CYP1A2, 2B6, 2C9 and 3A4 activity can be upregulated by their prototypical inducers (Antherieu et al., 2010; Kanebratt and Andersson2008; Lambert et al., 2009) and carcinogens and acetaminophen elicit
hepatotoxic responses, related to that of primary human hepatocytes (PHHs) (Jennen et al., 2010; McGill et al., 2011).

However, DMSO treatment increases cell death of the HepaRG cultures; the treatment induces a more than two-fold increase in cell leakage with loss of cell mass (Hoekstra et al., 2011), which may limit the sensitivity of hepatotoxicity tests. In addition, DMSO represses various hepatic functions, e.g. the elimination of galactose (Hoekstra et al., 2011), which may impair the usefulness of DMSO-treated HepaRG monolayers as a model for human liver.

Culturing HepaRG in 3D in a bioreactor, the AMC-bioartificial liver (AMC-BAL) for only 14 days without DMSO generated viable cultures mimicking the human liver to high extent (Nibourg et al., 2012): the HepaRG-AMC-BALs eliminated ammonia and lactate, and produced Apolipoprotein A-1 at rates comparable to freshly isolated hepatocytes. Moreover, CYP3A4 transcript level was high with 88% of the level of PHHs. The transcript levels of nuclear receptors hepatocyte nuclear factor 4 and pregnane X receptor, both central players in the regulation of drug metabolism (Tirona et al., 2003) were between 100-150% of human liver. Furthermore, a study in rats with acute liver failure showed that the HepaRG-AMC-BAL replaced the liver function leading to increased survival (Nibourg et al., 2012)

The aim of this study was to further characterize the HepaRG-AMC-BAL for the metabolism of endobiotics (testosterone, bilirubin, bile acids) and a xenobiotic (tolbutamide) to explore its suitability as a tool in compound safety studies. In addition, we studied the inducibility and localization of detoxification enzymes. Finally, we assessed bile acid production and composition, to determine the occurrence of hydroxylation, conjugation, and transport of bile acids, not only relevant for drug metabolism, but also to investigate the possibility that the HepaRG-AMC-BAL may serve as a model to study cholestasis-inducing activity of drugs.
Materials and Methods

**Biological materials and culture conditions**

HepaRG cells were kindly provided by prof. C. Guguen-Guillouzo (INSERM, Rennes, France). At day 0, laboratory models (9 mL) AMC-BALs were loaded with ~750 million cells and cultured in 500 mL recirculating HepaRG-CG medium, *i.e.* HepaRG medium without DMSO, but supplemented with 1 mM N-carbamoyl-L-glutamate (Sigma Aldrich, St. Louis, USA) (Nibourg *et al.*, 2012).

As a reference, PHHs were isolated at day 0 from liver tissue of female patients, aged 40 and 41 years, who underwent a partial hepatectomy as described (Hoekstra *et al.*, 2006). The procedure was in accordance with the Declaration of Helsinki and ethical standards of the institutional committee on human experimentation and after obtaining informed consent. PHHs were seeded in HepaRG-CG medium into 24-well Primaria™ plates (Becton Dickinson, Franklin Lanes, USA) at a cell density of 2.5 x 10^5 cells/cm². All monolayer cultures were kept at 37°C in a humidified atmosphere (95% air, 5% CO₂).

**Immunohistochemical analysis**

Transverse 8μm sections of formaline-fixed and paraplast-embedded BALs were obtained as described (Poyck *et al.*, 2007). Immunostaining for CYP3A4 was performed using a rabbit anti-human CYP3A4 antibody (1:100, Fitzgerald Industries International, Acton, USA) and alkaline phosphatase-labeled goat anti-rabbit immunoglobulin G (1:200, Sigma). Immunostaining for multidrug resistance-associated protein 2 (MRP2) was performed using mouse anti-MRP2 antibody M2II6 (Paulusma *et al.*, 2000) (1:200) and alkaline phosphatase-labeled goat anti-mouse immunoglobulin G (1:100, Sigma). For the immunostaining for bile salt export pump (BSEP) we used affinity purified rabbit anti-BSEP polyclonal antibody (1:100), which was kindly supplied by prof. B. Stieger, University Hospital, Zurich,
Switzerland. The reactions were developed with 5-bromo-4-chloro-3’-indolyphosphate p-toluidine and nitro-blue tetrazolium chloride (Roche, Penzberg, Germany).

**Detoxification activity tests**

At day 11 bilirubin glucuronidation capacity of HepaRG-AMC-BALs was measured (UGT1A1 activity). Subsequently, bioreactors were perfused with 500 mL recirculating HepaRG-CG medium in absence or presence of 10 µM rifampicin (Sigma). At day 14, the same HepaRG-AMC-BALs were tested for tolbutamide 4-hydroxylation (CYP2C9 activity) (test of 3 hrs), then reperfused with the 500 mL HepaRG-CG +/- rifampicine culture medium for 3 hrs and then tested for 6β-hydroxytestosterone (6β-OHT) production (CYP3A4 activity) during 2 hrs. The detoxification activity tests were performed by connecting the BALs to 40 mL culture medium including a single test compound, *i.e.* either 100 µM tolbutamide (Sigma), 125 µM testosterone (Sigma, 200x) or 10 µM bilirubin (Sigma). The BALs were flushed with 20 mL of the medium and the remaining medium was recirculated. Samples of 0.5 mL were taken at 15-60 min intervals during testing. Finally, we lysed the content of the bioreactors for protein content determination as described (Nibourg *et al.*, 2010).

For comparison, PHHs were tested at day 1. After washing the cultures twice with phosphate-buffered saline (PBS, Fresenius Kabi GmbH, Graz, Austria) we added 1 mL of HepaRG medium with single test components, as indicated above. Medium samples were taken at regular intervals during 1-2 hours of incubation. Finally, all test cultures were washed twice with PBS and stored at -20°C for protein determination.

All experiments with bilirubin were performed in the dark and all samples were immediately frozen.

**Test for bile salt production and conjugation**
Medium samples taken from day 14 HepaRG-AMC-BALs at t=0, 2, 8 and 24h of perfusion were assessed for total bile salt content and composition. The bile salt production rate was determined for the period between 0-2h; after that the production rate declined. In addition, PHHs in monolayer at day 1 were tested for total bile salt production. The cultures were washed 2-fold in PBS and incubated in 500 µL fresh HepaRG medium. Samples of 100 µL were taken at 2h intervals until 6 h after initiation of the test. During that period the bile salt production increased linearly. As indicated above, BAL and PHH cultures were subjected to protein determination after termination of the experiment.

*Biochemical assays*

Concentrations of bilirubin and its mono- and di-glucuronides were determined by high performance liquid chromatography (HPLC) as described before (Seppen et al., 1994; Seppen et al., 2006) with the following modifications; protein was precipitated by addition of two volumes of methanol (Merck, Darmstadt, Germany) and 1 minute centrifugation at 10,000 g. HPLC of deproteinized samples was performed on a Dionex (Amsterdam, the Netherlands) 3000 Ultimate HPLC, equipped with a pursuit C18 column (Agilent, Santa Clara, USA), variable wavelength detector set at 450 nM, and a flow rate of 1 mL/minute. For determination of 6β-OHT concentrations by ultra performance liquid chromatography (UPLC)-mass spectrometry, the deproteinized samples were first separated by Acquity UPLC (Waters, Milford, Massachusetts, USA) using an Acquity BEH C18, column, 1.7 um, 2.1 x 50 mm (Waters) with a linear elution gradient from 5% methanol (Biosolve, Valkenswaard, The Netherlands) in 0.1% formic acid (Merck) to 50% methanol in 0.1% formic acid at a flow rate of 0.6 mL/minute during 4 minutes. Detection of 6β-OHT was performed by the Xevo-TQS mass spectrometry system (Waters).

The 4-hydroxytolbutamide concentrations were measured by HPLC-tandem mass spectrometry. The system consisted of an AB Sciex (Framingham, USA) API3200 QTRAP
triple quadrupole mass spectrometer interfaced with an 1260 HPLC (Agilent). Chromatography was performed using a Zorbax Eclipse XDB C18 column (50 mm x 4.6 mm), at a flow rate of 1.5 ml/min. The mobile phase was ammonium acetate 5mM (Merck) in ultrapure water (A) and a mixture of acetonitrile (Merck) and methanol (50/50; v/v) with 0.3% of formic acid (B). The proportion of the mobile phase B was increased linearly from 5 to 55% in 2.5 min, and then the column was flushed with 98% of the mobile phase B and allowed to re-equilibrate at the initial conditions. The total run time was 3.9 min.

Total bile salt concentrations were assessed by using the Total Bile Acids Assay Kit (Diazyme Laboratories, Poway, USA). Bile salt composition was determined by HPLC electrospray tandem mass spectrometry (Bootsma et al., 1999). Total protein concentrations were measured using the Coomassie Brilliant Blue G-250 staining kit (Bio-Rad, Hercules, USA).

Metabolic rates were established by calculating the concentration changes in time and were corrected for protein content per BAL or per well. The total cell mass is stable under the given bioreactor culture conditions (Nibourg et al., 2012), and therefore the bilirubin glucuronidation data obtained from HepaRG-AMC-BALs at day 11 were normalized for protein content of the cell lysate of HepaRG-AMC-BALs at day 14.

Statistical analysis

Student’s t-tests were used to determine statistical differences. Significance was reached if $P < 0.05$. SPSS 12.0.1 (SPSS Inc., Chicago, USA) was used for statistical analysis. Average values ($\pm$ standard deviation) are reported.
Results

High level of polarization in heterogenous BAL culture

Immunohistochemical stainings of BAL cultures harvested at day 14 show that the HepaRG cells are viable and primarily located in the matrix of the bioreactor, circularly wound around a core. The cells heterogeneously expressed CYP3A4, as the intensity of cytoplasmic CYP3A4 staining varied clearly (Fig. 1 A, B). Furthermore, we did not observe a clear pattern of CYP3A4 positive clusters amidst CYP3A4-negative cells, as found for +DMSO monolayer (Cerec et al., 2007).

MRP2, a canalicular membrane transporter (Bohan and Boyer2002), was expressed in the canalicular structures throughout the BAL culture (Fig. 1 C, D), indicating that the HepaRG cells were organized into a highly polarized and liver-like structure. The expression of BSEP, the canalicular membrane transporter of bile acids was also confined to canalicular structures, but at very low frequency (Fig. 1 E, F).

Substantial phase 1 and phase 2 drug metabolism in the HepaRG-AMC-BAL

As phase 1 drug metabolism markers, basal and rifampicine inducible CYP2C9 and CYP3A4 activities were tested in 14-day BAL cultures by measuring the hydroxylation of tolbutamide and testosterone, respectively (n=3/group) (Fig. 2A,B). The CYP2C9 activity was $0.26 \pm 0.07 \text{ nmol/h/mg protein}$, 240% the rate of PHHs (historical data). The induction of rifampicin was not significant, although a trend towards increased CYP2C9 activity was observed (Fig. 2A). The testosterone 6β-hydroxylation, was two-fold increased by rifampicin (Fig. 2B). The basal activity was $11.2 \pm 4.0 \text{ nmol/h/mg protein}$, 37% the rate of PHHs (historical data) (Nibourg et al., 2012).

As a marker for the phase 2 enzyme UGT1A1, the rate of bilirubin glucuronidation was tested at day 11 of the BAL culture (Fig. 2C, n=6). As a reference, bilirubin glucuronidation was compared with the activity in PHHs. (n=4 for 2 isolates). Production rates of bilirubin
mono-glucuronides and di-glucuronides were 44% and 15%, respectively in the BAL compared to PHHs, yielding 26% of total bilirubin glucuronidation activity.

**Secretion of and conjugation of bile salts in the HepaRG-AMC-BAL**

We performed a quantitative measurement of bile salt secretion and a qualitative assessment of bile salt conjugation. The bile salt secretion of the HepaRG-AMC-BALs (n=4) was 43% of PHH cultures (n=3-4 for 3 isolates) (Fig. 3A).

The accumulation and conjugation of specific bile salts was qualitatively determined by comparison of the HPLC mass spectra of bile salts in medium samples (Fig. 3B). The analysis was, however, hampered by the accumulation of unidentified bile acids that probably arose from the metabolism of bovine bile salts from the HepaRG medium which contains 10% (v/v) fetal bovine serum. As an example, in the chromatogram of trihydroxy bile salts we observed a stable peak for cholic acid (CA), representing a concentration of 0.05 μM and an unidentified (NI), probably non-human, bile salt peak accumulating during 24h until a concentration of 0.26 μM (Fig. 3C). The latter observation indicates that uptake and hydroxylation of bile salts occurred. Of the taurine-conjugated trihydroxy bile acids, tauro-CA (t-CA) increased from 0.1 to 0.3 μM and again an unidentified taurine-conjugated trihydroxy bile acid accumulated until a concentration of 0.1 μM. This indicates the occurrence of bile acid amidation. Of all conjugated bile salts, the accumulation of t-CA was most prominent (Fig. 3D), finally making up 20% of the total bile acid pool at 24h of BAL culture. The secretion of dihydroxy bile salts and the conjugation of a glycine-moiety to the bile acids was negligible (data not shown).
Discussion

The data presented here show that the HepaRG cells in the AMC-BAL organize within 2 weeks into a highly polarized bile acid producing liver-like tissue with substantial drug metabolism, ranging from 26% (bilirubin) to 240% (tolbutamide) of PHHs. These high levels of drug metabolism have been achieved in HepaRG cells without the usage of DMSO, yielding the HepaRG-AMC-BAL a promising tool for drug safety studies targeted to the liver. The HepaRG-AMC-BALs take up, produce, metabolize, and efflux bile acids, with a rate of bile acids accumulating in the medium of 43% of PHHs. Bile acid production and efflux is essential for hepatic signaling and for driving the bile flow (Kosters and Karpen2008). Our results open the possibility to apply the HepaRG-AMC-BAL for studying the excretion of drugs or their metabolites via bile and for testing the cholestasis-inducing activity of drugs. A well-known target of drugs that induce cholestasis is BSEP (Gerloff et al., 1998). Under the given experimental conditions, BSEP expression was low in the HepaRG-AMC-BALs. It is, therefore likely that bile acids were exported via sinusoidal transporters as Mrp3, Mrp4 and the organic solute transporter α/β (Kosters and Karpen2008). However, this needs to be further confirmed, in addition to the structure of the canaliculi in the bioreactor culture, which will also determine the destination of the bile acids. Notably, the low BSEP expression, also observed in HepaRG monolayer cultures, may be drastically upregulated by pretreatment with chenodeoxycholic acid (Le Vee et al., 2006). Therefore such a pretreatment may have marked effects on the transport of bile acids and enhance the applicability of the HepaRG-AMC-BAL for testing cholestasis-inducing effects of drugs.

The HepaRG-AMC-BAL has the capacity to hydroxylate bile acids, which is a CYP3A function, and to conjugate them with taurine, which is catalyzed by the enzymes bile acid CoA synthetase and bile acid-CoA:amino acid N-acetyltransferase (Trottier et al., 2006). The latter two enzymes are controlled by nuclear receptors farnesoid X receptor and peroxisome proliferator-activated receptor alpha (Trottier et al., 2006). These two transcription factors
are also thought to play a role in the coordinate expression of UGTs and hepatic transporters, as basic elements of hepatic drug metabolism. Together with the high expression of hepatocyte nuclear factor 4 and pregnane X receptor, previously shown in the HepaRG-AMC-BAL, this suggests that major nuclear receptors co-ordinately regulating hepatic drug metabolism are functional in the HepaRG-AMC-BAL.

The hepatic differentiation promoting activity of the AMC-BAL is probably induced by the medium flow and the 3D organization of the culture and perhaps by the high oxygenation on site. It has been generally established that these three factors contribute to the maintenance of functionality of primary hepatocyte cultures and/or HepaRG cells, and more specifically to their drug metabolism (Landry et al., 1985; Legendre et al., 2009; Poyck et al., 2008; Tilles et al., 2001).

Recently, the HepaRG cells have been cultured in 3D in another bioreactor with perfusion of medium and oxygen supply on site (Darnell et al., 2011). When applying a >3 weeks BAL culture protocol including DMSO treatment prior to the test (not during the test), the cells showed clear drug metabolism, but no further upregulation of hepatic functions was established and even lactate production was found, whereas differentiated hepatocytes usually consume lactate (van de Kerkhove et al., 2005). Thus, culturing in 3D, with medium perfusion and oxygen supply on site is not per se sufficient for further promoting the differentiation of HepaRG cells. This may very well be attributable to the negative effects of DMSO. In a follow-up experiment DMSO was omitted in their bioreactor culture protocol (Darnell et al., 2012). The bioreactor, cultured for 5 days, was capable of biotransforming diclofenac and drug AZD6610, however the rates of substrate clearance and metabolite formation were lower compared to day 0 suspensions, indicating that their culture protocol or bioreactor still requires further improvement.

Admittedly, some characteristics of the HepaRG-AMC-BAL are also still low compared to that of PHHs, e.g the CYP1A2 expression (Nibourg et al., 2012) and inducibility of CYP2C9
and CYP3A4. To value the HepaRG-AMC-BAL as a tool for drug safety testing, we further need to explore experimental conditions, such as duration of induction, concentration of the inducers, and prolonged (exceeding 14 days) culture times of HepaRG-AMC-BALs. The latter is specifically interesting, as CYP1A2, 2B6 and 3A4 transcript levels and CYP3A4 activity increase further after 2 weeks of BAL culturing (Nibourg et al, data not shown). In addition the metabolism of an extended set of probe substrates and the response to hepatotoxins still needs to be addressed.

On the other hand, application of the HepaRG-AMC-BAL for drug safety studies holds great promise, not only for its high and all-round hepatic functionality, but also for its long-term functionality, allowing chronic exposure experiments for >2 weeks. In addition, the medium volume can be varied over a large range, which enables either very rapid experiments (with high cell/medium ratio) or, on the other hand, chronic exposure experiments (with low cell/medium ratio) with low concentrations of drugs with high hepatic metabolism.

Concluding, culturing HepaRG cells in the AMC-BAL yields substantial phase 1 and phase 2 drug metabolism, while maintaining high viability. Therefore, and for the high flexibility in experimental set-up, HepaRG-AMC-BALs seem suitable for testing metabolism and toxicity of drugs.
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References


of the multidrug resistance protein 2 during bile duct ligation in rat liver. *Hepatology* 31:684-693.


Footnotes

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Figure legends

Figure 1. CYP3A4 (A and B), MRP2 (C and D) and BSEP (E and F) stainings of cross sections of HepaRG-AMC-BALs at 14 days of culture at two different magnifications. The full transverse sections (A, C and E, bars: 500 µm) show the spirally wound matrix layers between gas capillaries (arrows show spaces left after washing off capillaries by experimental procedure) in between. Details of the matrix (B, D and F, bars: 10 µm) show the matrix fibers (arrowheads) with HepaRG cells with heterogeneous CYP3A4 expression and canalicular MRP2 and BSEP expression.

Figure 2. Phase 1 and phase 2 drug metabolic activity of the HepaRG-AMC-BALs. A) the accumulation of hydroxylated tolbutamide (CYP2C9 activity) in medium at BAL culture day 14 after a 3-days induction with 10 µM rifampicin (black squares; control without rifampicin, white squares), B), the accumulation of 6β-OHT (CYP3A4 activity) in medium at BAL culture day 14 after a 3-days induction with 10 µM rifampicin (black squares; control without rifampicin, white squares), C) the production of mono, di and mono+di glucuronides of bilirubin of HepaRG-AMC-BALs at day 11 (grey bars) and of PHH monolayers (day 1, white bars), with the % relative to PHH monolayers given in the bars. Significance: * \(p < 0.05\) versus no rifampicin group, \(\Doteq p < 0.05\) versus PHHs.

Figure 3. Secretion and composition of bile acids by HepaRG-AMC-BALs at day 14. A) Production of bile acids of HepaRG-AMC-BALs and of PHH monolayers (day 1), with the % relative to PHH monolayers given in the bar. Significance: \(\Doteq p < 0.05\) versus PHHs. B) the accumulation of four identified bile acids in the medium, C and D) chromatograms of unconjugated trihydroxy bile salts (C) and taurine-conjugated trihydroxy bile salts (D) of the culture medium at t=0 and t=24h. Large peaks are indicated with their concentration.
Figure 1
Figure 2
Figure 3