Comparison of Culture Media for Bile Acid Transport Studies in Primary Human Hepatocytes

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Background: **Primary human hepatocytes are a useful *in vitro* model system to examine hepatic biochemical pathways, liver disorders and/or pharmacotherapies. This system can also be used for transport studies to investigate uptake and excretion of bile acids. Proper modeling of hepatic function requires careful attention to media components, and culture substrates and conditions.** ***Objectives:*** **To examine the effects of different culture media and conditions on bile acid transport in cultured human hepatocytes.** ***Methods and Results:*** **Hepatocytes cultured in Williams' medium E showed an increase in both uptake and excretion of taurocholate compared to cells cultured in Dulbecco's Modified Eagle Medium (DMEM). Supplementation of DMEM with glutathione or ascorbic acid did not compensate for the lower transport. The difference can be explained by lower mRNA expression of the transporter proteins sodium taurocholate cotransporting polypeptide (NTCP) and bile salt export pump (BSEP; ABCB11) when cultured in DMEM. Hepatocytes cultured in DMEM also display fewer and smaller bile canaliculi. Following extended time in culture supplementation of Williams' medium E with dexamethasone increased the expression of NTCP and BSEP.** ***Conclusion:*** **Williams' medium E is superior to DMEM for transport studies in primary human hepatocytes. Supplementation with dexamethasone increase mRNA levels of NTCP and BSEP. (J Clin Exp Hepatol 2012;2:315–322)**

The study of *in vitro* transport of bile acids in hepatocytes provides a useful method for analyzing drug disposition, biliary clearance and different conditions causing cholestasis.

Under normal conditions bile acids are taken up by hepatocytes from portal circulation by high affinity sodium-dependent, sodium taurocholate cotransporting polypeptide (NTCP) and to a lesser extent by the sodium-independent organic anion-transporting polypeptides (OATPs) in the sinusoidal membrane.[1,2] Within the hepatocyte, returning bile acids are mixed with newly synthesized bile acids and are secreted into bile. Conjugated bile acids are secreted from the hepatocyte canalicular membrane into bile by the bile salt export pump (BSEP; ABCB11) which is an adenosine triphosphate-binding cassette transporter. A small amount is also secreted by multidrug resistance protein 2 (MRP2).[1,2]

In cholestasis, bile flow from the liver to the intestine is impaired and as a consequence toxic bile acids and other metabolites are retained within the hepatocyte. In children, cholestasis with early onset accounts for a large proportion of the cases with severe liver disease with high mortality and morbidity. Cholestasis in infancy has many different causes and the etiology is unknown in 20–25% of affected patients.[3] The disease can be caused by mutations in transporter proteins. For example, mutations in the ABCB11 gene, encoding for BSEP, are associated with progressive familial intrahepatic cholestasis type 2.[4] Acquired cholestasis may be due to infections, drug-induced hepatocellular injury or total parenteral nutrition.[5,6] To study the possible subtle difference between normal and diseased livers one needs to first investigate how bile acids are transported in liver cells from normal tissue. Thus, in order to use hepatocytes as a model system for cholestasis culture conditions that allow for bile acid transport in cultured cells have to be attained. Previous reports have investigated effects of different conditions for bile acid transport in rat and mouse hepatocytes[7–9] but the knowledge on how different culture conditions affect bile acid transport in human hepatocytes is limited.

Two commonly used culture media for hepatocytes are Dulbecco's Modified Eagle Medium (DMEM) and

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Abbreviations: DMEM: Dulbecco's Modified Eagle Medium; NTCP: sodium taurocholate cotransporting polypeptide; BSEP: bile salt export pump; OATP: organic anion-transporting polypeptide; MRP2: multidrug resistance protein 2; WE: Williams' medium E; HBSS: Hank's Balanced Salt Solution; CgamF: cholylglycylamido-fluorescein; GSH: glutathione; AA: ascorbic acid
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Williams' medium E (WE), in this report we examined how bile acid transport is affected by culturing hepatocytes in these media. While there are many small differences between the components of DMEM and WE, two substantial differences are the presence of ascorbic acid and glutathione in WE. We therefore supplemented DMEM with these compounds to investigate if bile acid transport was affected. Dexamethasone is a synthetic glucocorticoid frequently included as a supportive or protective factor in hepatocyte culture media and even in flushing and transport solutions used to cold-preserve livers for transplantation. Since dexamethasone is such a common additive to culture media, its effects on transport activity in cultured hepatocytes were investigated.

The aim of the present study was to investigate the influence of different culture media, media supplements and time in culture for activity and expression of bile acid uptake and efflux transporters NTCP and BSEP in cultured healthy human hepatocytes.

MATERIALS AND METHODS

Isolation of Primary Human Hepatocytes

Normal human liver tissue was obtained from patients (n = 11, Table 1) undergoing surgical liver resection due to cancer or from donor livers that could not be used for transplantation. Approval to use parts of resected human liver specimens for research was given by the Ethics Committee at Karolinska Institutet and from the Institutional Review Board at University of Pittsburgh.

Hepatocytes were isolated by a three-step perfusion technique, utilizing EGTA and collagenase, as previously described by Strom et al[10] and updated by Gramignoli et al[11] 1.5 million cells were plated onto 6 well culture dishes precoated with collagen I (3.3 mg/ml), after 24 h cells were over laid with matrigel (0.233 mg/ml) to further polarize the cells. Hepatocytes cultured in this sandwich configuration develop functional canalicular spaces between cells which allow transport studies. In all experiments, hepatocytes were cultured in cell culture media supplemented with amphoteracin, gentamicin and insulin (120 nM). In the first experiments cells were cultured in WE, WE and DMEM in equal parts, DMEM or in DMEM supplemented with glutathione (0.16 μM) or ascorbic acid (11 μM). In all subsequent experiments cells were cultured in WE without or with the addition of dexamethasone. The medium was changed 1 h after plating and then daily until harvesting. Following 17, 72 or 96 h in culture bile acid transport was measured and cells were harvested in Trizol for quantification of mRNA.

Table 1 Demographics.

Liver number	Age	Gender	Diagnosis
HH1261	18	M	Neuroendocrine tumor
HH1270	77	F	Deceased donor
HH1274	55	F	Deceased donor
HH1281	16	M	Deceased donor
HH1571	62	F	Liver resection due to metastatic colon carcinoma
HH1591	12	F	Deceased donor
HF79	39	M	Liver resection due to suspected tumor in Chron's disease
HF82	49	F	Liver resection due to metastatic pancreatic carcinoma
HF83	69	F	Deceased donor
HF133	76	M	Colon carcinoma
HF134	50	M	Deceased donor
VF1	50	M	Deceased donor

F, female; M, male.

Bile Salt Transport Assay

Bile salt transport was measured by using ^{3}H-Taurocholate as described previously[12] with some minor changes, see Figure 1 for a cartoon illustrating the method. First, cell culture media was collected and cells washed with Hank's Balanced Salt Solution (HBSS) + (HBSS supplemented with $CaCl_2$ (anhyd.), $MgCl_2 \times 6\ H_2O$, $MgSO_4 \times 7\ H_2O$) and incubated at 37 °C. The buffer was aspirated and cells loaded by adding HBSS+ containing non-labeled taurocholate and ^{3}H-Taurocholate and incubated at 37 °C for 20 min. The cells were washed with ice cold HBSS+. Half of the wells received HBSS+ and the other half HBSS− (HBSS supplemented with EGTA) and the cells were then incubated for another 20 min at 37 °C. Aliquots of media were obtained from each well and counts per minute analyzed with a beta counter. The remaining fluid was quickly aspirated and the cells were lysed in buffer containing NaOH/SDS. Lysates were used for counting radioactivity and protein determination.[13]

Specific activity for uptake was defined by the sum of both effluxed and retained radioactivity in HBSS+ samples. Since desmosomes between the cells break in absence of Ca/Mg allowing the radioactivity to efflux, specific activity for canalicular transport was defined by the difference in efflux of ^{3}H-Taurocholate in the absence and presence of Ca/Mg. To normalize different efflux capacities between different livers, efflux from each treatment was divided by total efflux for that liver.

Visualization of Bile Canaliculi

Bile canaliculi was visualized by treating cells cultured in DMEM or WE with cholylglycylamido-fluorescein (CGamF), a generous gift from Dr. Alan Hofmann, San Diego. CGamF is a fluorescein-labeled bile acid analog transported by NTCP and BSEP. Cells were washed as described for the transport assay and incubated with