Addition of Dexamethasone Alters the Bile Acid Composition by Inducing CYP8B1 in Primary Cultures of Human Hepatocytes

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Background: **Primary human hepatocytes offer the best human *in vitro* model for studies on human liver cell metabolism. Investigators use a variety of different media supplements and matrix biocoatings and the type of culture system used may influence the outcome.** ***Objectives:*** **To optimize *in vitro* conditions for primary human hepatocytes with regard to bile acid synthesis.** ***Methods:*** **Human hepatocytes were isolated and cultured on collagen type I or EHS matrigel in cell media with or without dexamethasone. The glucocorticoid receptor (GR) antagonist RU486 was used to elucidate the involvement of GR.** ***Results:*** **Hepatocytes cultured on EHS matrigel produced more bile acids and expressed higher levels of cholesterol 7α-hydroxylase (CYP7A1) than cells cultured on rat tail collagen. Supplementation with dexamethasone increased the formation of cholic acid (CA) and decreased chenodeoxycholic acid formation. In line with these results, the mRNA expression of sterol 12α-hydroxylase (CYP8B1) increased following dexamethasone treatment. Surprisingly, the mRNA expression of CYP7A1 and CYP27A1 was not increased to the same extent. By using the GR antagonist RU486, we concluded that CYP8B1 induction is mediated *via* a GR-independent pathway. An altered expression of retinoid-related orphan receptor (ROR) α and ROR α target gene Glucose-6-phosphatase (G6Pase) suggests that ROR α signaling may regulate CYP8B1 expression.** ***Conclusion:*** **Primary human hepatocytes have an increased bile acid synthesis rate when cultured on matrigel as compared to collagen. Exposure to glucocorticoid hormones stimulates the expression of CYP8B1, leading to an increased formation of CA and alteration of the bile acid composition. The effect is most likely mediated through a GR-independent pathway, possibly through ROR α. (J Clin Exp Hepatol 2016;6:87–93)**

Production of bile is one of the many important functions of the liver. Disruption of this process, called cholestasis, causes considerable morbidity and need of medical attention. Good research tools to study bile acid metabolism are therefore important.

In humans, two primary bile acids are synthesized: cholic acid (CA) and chenodeoxycholic acid (CDCA). These bile acids are produced by two major pathways, the neutral pathway and the acidic pathway.[1,2] The initial step of the neutral pathway is a hydroxylation at the 7α-position of cholesterol, a reaction catalyzed by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1). The acidic pathway begins with the oxidation of the cholesterol side chain, in which the first step is catalyzed by sterol 27-hydroxylase (CYP27A1). The enzyme responsible for determining the relative amounts of CA and CDCA in both pathways is sterol 12α-hydroxylase (CYP8B1). The ratio between the bile acids is important for feedback regulation of bile acid synthesis.[3] It is also important for both cholesterol absorption and accumulation of cholesterol esters in the liver, as well as for gallstone formation.[4–6]

Many *in vitro* studies on human bile acid synthesis have been performed in HepG2 cells. However, HepG2 cells have limitations since these cells leak bile acid precursors and unconjugated bile acids that are not secreted by the liver under normal conditions.[7–10] The first reports on cholesterol conversion to bile acids in primary hepatocytes were from rat studies.[11,12] We have found that primary human and rat hepatocytes in culture exhibit significant species differences in both types of bile acids formed and, more importantly, in the regulation of bile acids homeostasis.[13] We have also shown that primary human hepatocytes conjugate all secreted CA and CDCA by amidation to

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Abbreviations: BSEP: bile salt export pump; CA: cholic acid; CDCA: chenodeoxycholic acid; CYP7A1: cholesterol 7α-hydroxylase; CYP8B1: sterol 12α-hydroxylase; CYP27A1: sterol 27α-hydroxylase; FXR: farnesoid X receptor; G6Pase: glucose-6-phosphatase; GR: glucocorticoid receptor; NTCP: Na^+-taurocholate cotransporting polypeptide; PXR: pregnane X receptor; ROR: retinoid-related orphan receptor
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glycine or taurine[13,14] and thus, no unconjugated bile acids are found in the media.

Bile acid synthesis is affected by signaling from many different pathways including those from nuclear hormone receptors, such as FXR, pregnane X receptor (PXR), and signals derived from the extracellular matrix. There are numerous reports on the *in vitro* regulation of mRNA levels of enzymes important for bile acids synthesis, such as CYP7A1 and CYP8B1,[15–19] but experimental data are not always consistent. This may be due to the different culture conditions used by investigators. Both the cell substrate and the composition of the culture media may affect the ability to produce bile acid. Thus there is a need for further investigation of how different commonly used culture conditions alter the production of bile acids. Even though culturing hepatocytes on matrigel or in a matrigel sandwich results in a more adequate cell morphology, the effect on P450 expression in human cells has been disputed. It has been reported that the expression of P450 enzymes is not changed and that the expression may be dependent on plating density rather than on the type of extracellular matrix used.[20,21] Since dexamethasone is a common additive to culture media, we have previously investigated their effect on bile acid transporters in primary hepatocytes and shown that dexamethasone addition in the cell culture media increased the expression of BSEP and NTCP.[22] In this study, we investigated how different substrates, collagen and matrigel, and the addition of dexamethasone to the culture media influence bile acid synthesis and secretion.

PATIENTS AND METHODS

Isolation of Primary Human Hepatocytes

Normal human liver tissue was obtained from patients (n = 19) 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 local Ethics Committee in Stockholm alternatively from the Institutional Review Board at University of Pittsburgh, see Table 1, for information on liver tissue donors. Hepatocytes were isolated by a three-step perfusion technique, utilizing EGTA and collagenase (collagenase XI from Sigma), as previously described by Strom et al.[23] The hepatocytes were plated onto cell culture dishes precoated either with rat tail collagen type I or EHS matrigel. Hepatocytes were cultured under standard conditions in William's E medium supplemented with 12 nM insulin, amphotericin (250 µg/ml), and gentamicin (50 mg/ml). 1.5 million cells were plated onto 6-well plates and cultured in cell media with or without the addition of 100 nM dexamethasone. Untreated cells from the same liver always served as controls to the treated cells. In Figures 4 and 5B, cells were also treated with 1 µM RU486. The medium was changed one hour after plating and then daily until harvesting. On day 5, cells were harvested in Trizol for quantification of specific mRNAs, and cell culture medium was analyzed for bile acids.

Table 1 List of Livers Used in the Experiments. F = female, M = male.

Liver	Age	Gender	Diagnosis
HF79	39	M	Hepatocellular carcinoma
HF82	47	F	Pancreatic cancer
HF83	69	F	Donor
HF107	64	F	Colon cancer
HF108	45	F	Crm
HF109	57	F	Colon cancer
HF110	73	M	Colon cancer
HF111	65	F	Liver cancer
HF113	62	F	Colon cancer
HH1436	49	M	Colon cancer
HH1437	50	F	Adeno cancer
HH1438	38	F	Focal nodular hyperplasia
HH1439	68	M	Focal nodular hyperplasia
HH1465	76	M	Hepatocellular carcinoma
HH1467	52	F	Colon cancer
HH1468	38	M	Colon cancer
HH1469	46	F	Donor
HH1571	62	F	Colon cancer
HH1591	12	F	Donor

Real-Time PCR

RNA was isolated using Trizol reagent (Invitrogen, Stockholm, Sweden) and cDNA synthesis was performed using MultiScribe Reverse Transcriptase (Applied Biosystems, Stockholm, Sweden). mRNA expression was quantified with Quantitative real-time PCR using Taqman probes from ABI, and analysis was performed on an ABI Prism 7000 instrument (Applied Biosystems, Stockholm, Sweden). As endogenous control cyclophilin was used.

Analysis of Bile Acids

Bile acids in cell culture media were analyzed as described previously.[13] Briefly, 500 ng of deuterium-labeled CA and CDCA was added to 1 ml of cell culture medium. The mixture was hydrolyzed in 1 M KOH at 120 °C overnight. The samples were extracted with ethyl ether, and following acidification with hydrochloric acid to pH 1, the samples were extracted again with ethyl ether. The samples were washed until neutral, evaporated and methylated with trimethylsilyldiazomethane and derivatized using hexamethyldisilazane and trimethylchlorosilane in pyridine. Samples were analyzed by gas chromatography/mass