EXPRESSION OF LIVER-SPECIFIC CYTOCHROME P450 ISOENZYMES AND OXYGENASES IN C3A CELLS PRIOR TO AND AFTER TREATMENT WITH THE ELAD LIVER SUPPORT SYSTEM

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EASL The Home of Hepatology
50th THE INTERNATIONAL LIVER CONGRESS 2015
APRIL 22-26, VIENNA, AUSTRIA

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BACKGROUND

Acute-on-chronic liver failure (ACLF) and acute liver failure (ALF) are characterized by impaired liver function, multi-organ failure, and high short-term mortality. Cell-based support of liver function could therefore be of therapeutic benefit if the cell therapy was demonstrated to provide metabolic functions required of normal hepatocytes, such as detoxification. The ELAD System (Fig. 1c) provides extracorporeal exposure to human hepatoblastoma-derived C3A cells and is under evaluation in several clinical trials for various liver disease indications.

OBJECTIVES

The aims of this study were to characterize the expression levels of liver-specific cytochrome P450 isoenzymes (CYP) and oxygenases in ELAD C3A cells during clinical product production and after use in clinical treatment.

MATERIALS & METHODS

C3A cells were harvested as monolayer cultures (Fig 1a), from ELAD C3A hollow-fiber cartridges at production maturity (Fig 1b), or after 5 days of clinical use in alcohol-induced liver disease (AILD) subjects (Fig 1c).

Monolayer C3A cells (n=4) cultured in proprietary MM Media (Hyclone) were harvested for mRNA isolation (Qiagen RNeasy) and analyzed on TaqMan Human CYP450 and Oxygenases Array Plates (ABI); however, not all array gene targets are liver-specific.

ELAD C3A hollow-fiber cartridges were recovered at production maturity (n=5), and after 5 days of clinical use (n=9) and processed as above. Gene expression levels vs. controls were calculated by the ΔΔCt method using the GUSB gene as an endogenous control. The relative expression of each gene was determined as fold increase or decrease relative to the average expression in monolayer cells using the formula: Relative Expression = $2^{-\Delta\Delta Ct}$.

RESULTS

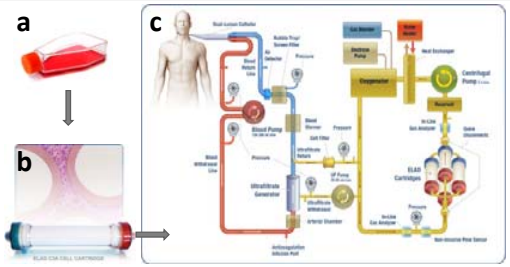

Figure 1. C3A cells were harvested as monolayer cultures (a), from ELAD C3A hollow-fiber cartridges at production maturity (b), or after 5 days of clinical use in alcohol-induced liver decompensation (AILD) subjects (c).

Over 60 gene targets evaluated were detected in C3A cells. Among these included CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 (Fig. 2), which are collectively responsible for metabolizing nearly 90% of all drugs [1] as well as other CYPs involved in detoxification.

Up-regulation in ELAD cartridge C3A cells vs. monolayer was observed for CYP11B2, CYP17A1, CYP21A2, CYP2A6, CYP2A7, CYP2E1, CYP3A7, CYP4A11/CYP4A22, CYP51A1 and CYP7A1 (Fig. 3), whereas CYP19A1, CYP24A1, CYP2S1, NENF and SC4MOL were down-regulated (Fig. 4).

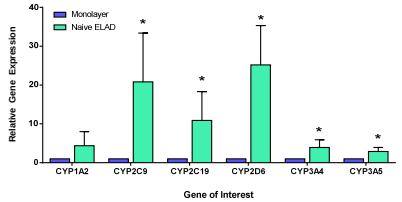

Figure 2. All of the major drug-metabolizing CYPs (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5) are expressed in monolayer and ELAD C3A cells (* p<0.05 ELAD vs. monolayer, Student's t test).

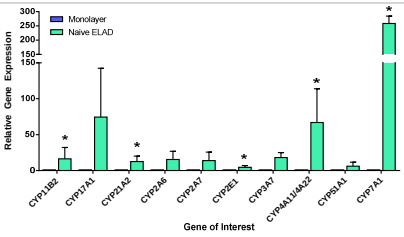

Figure 3. Several other CYPs are up-regulated in ELAD C3A cells relative to monolayer C3A cells (* p<0.05 vs. monolayer, Student's t test).

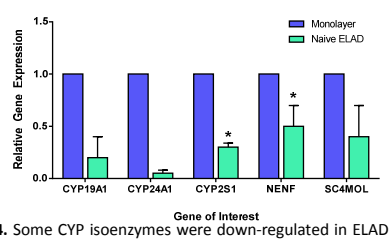

Figure 4. Some CYP isoenzymes were down-regulated in ELAD C3A cells relative to monolayer C3A ce3lls (*p<0.05 vs. monolayer, Student's t test).

Up-regulation in ELAD C3A cells prior to vs. after AILD subject treatment was observed for CYP11B2, CYP2A6, CYP2A7, CYP2C19 and CYP1A2, whereas down-regulation was noted for and CYP17A1 (Fig. 5). Apparent down-regulation of TYR and CYP51A1 disappeared with additional clinical sample replicates.

C3A cells from AILD subject-recovered ELAD cartridges exhibited differences in up- or down-regulation between individual subjects among 16-22 different gene targets (Fig. 6).

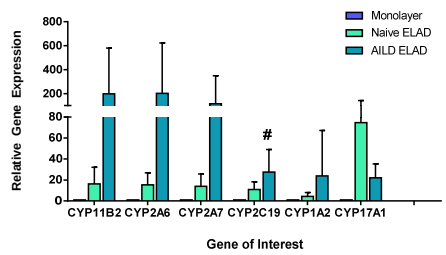

Figure 5. Several CYPs were up-regulated in ELAD C3A cells after AILD subject treatment (# p=0.06, Student's t test), whereas CYP17A1 was down-regulated subsequent to subject exposure.

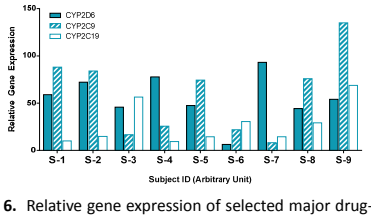

Figure 6. Relative gene expression of selected major drug-metabolizing CYPs in ELAD C3A cells subsequent to use in treatment of nine different AILD subjects shows variable expression from subject to subject.

SUMMARY

ELAD C3A cells express a variety of cytochrome P450 isozymes including those most involved in drug metabolism such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5. The mRNA expression of these CYPs and several others increases when the C3A cells are cultured three-dimensionally within the ELAD cartridges relative to monolayer culture.

The C3A cells in the ELAD System show different and varied expression patterns after exposure to subject treatment. It remains to be shown whether or not these changes correlate with functional changes in subjects' metabolic intermediates.

CONCLUSIONS

This study provides supporting evidence that the C3A cells comprising the ELAD System exhibit a diverse expression of various critical liver metabolic enzymes, and is supportive of previous studies using drug metabolites as evidence of the presence of selected CYP isoenzymes.

Culturing the C3A cells three-dimensionally within the ELAD cartridges enhanced the overall pattern of CYP expression and illustrates how organizational structure may positively benefit metabolic function.

It also illustrates that unique responses can be observed in cell-based therapies as a result of dynamic interactions with an individual subject's unique physiology.

These data may help clarify the mechanism of action of the ELAD System in supporting detoxification and potentially improving survival in subjects with ACLF and ALF.

ACKNOWLEDGMENTS

1.Gilman, G., *The Pharmacological Basis of Therapeutics*. Eleventh ed. 2006: McGraw-Hill.

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