addition, Huh-7 hepatocytes exposed to toxic concentrations of LCFA (a mixture of oleic and palmitic acid) for 24 hours were treated with L-FABP inducers clofibrate or simvastatin (6.25-100 mM) for a subsequent 48 hours prior to documenting cell toxicity.

RESULTS: Neither β-estradiol, progesterone or a combination thereof consistently decreased LCHAD mRNA or protein expression. Moreover, hepatocyte survival was not altered by either clofibrate or simvastatin.

CONCLUSIONS: Increases in steroid hormones associated with pregnancy are unlikely to contribute to LCFA-induced hepatotoxicity in LCHAD deficient women. Induction of L-FABP does not hold promise as a therapeutic strategy for pregnant women with AFLP or HELLP.

Key words: LCHAD; AFLP; HELLP; Long-chain fatty acids; Pregnancy; Liver failure; Hepatitis

INTRODUCTION

Acute fatty liver of pregnancy (AFLP) and the Hemolysis Elevated Liver Enzymes and Low Platelet (HELLP) Syndrome are rare but serious conditions that tend to occur in the third trimester of pregnancy. The incidence of AFLP has been estimated to be...
Effect of L-FABP Inducers on Cell Survival

Human Huh-7 hepatocytes (1×10^4 cells/well) were seeded in 96 well plates and pre-treated with a 1:1 mixture of the LCFA oleic acid and palmitic acid (0.55 mM) for 24 hours. Thereafter, clofibrate or simvastatin (6.25-100 nM) were added (with continued exposure to LCFA) for an additional 48 hours prior to documenting cell viability by WST-1 analysis. Wells with no clofibrate or simvastatin served as controls.

Data Analyses

All experiments were performed on at least three occasions and the results provided represent the mean ± SEM unless otherwise indicated. Statistical analyses consisted of a Student’s T-test for parametric data and Wilcoxon Rank-Sum test for non-parametric data. P values <0.05 were considered significant.

RESULTS

The results of LCHAD mRNA expression in B2325 cells following exposure to β-estradiol alone, progesterone alone and a combination of the two steroids for 0-72 hours are provided in figure 1. Although β-estradiol markedly inhibited LCHAD mRNA expression at 24 hours, by 48 and 72 hours, expression levels had returned to baseline values. Moreover, there was no concentration-dependent effect of β-estradiol on LCHAD mRNA expression (Figure 2). As with β-estradiol, there was a trend towards progesterone inhibiting LCHAD mRNA expression at 24 and 48 hours but this effect was no longer apparent at 72 hours. Of note, these effects at 24 hours for β-estradiol and 24-48 hours for progesterone were not apparent when the combination of β-estradiol and progesterone was employed.

In keeping with a lack of significant and consistent inhibition of LCHAD mRNA by either steroid hormone alone or combination thereof, were the results of Western blotting for LCHAD protein expression. Here, at no time following exposure to β-estradiol and/or progesterone was LCHAD protein expression consistently decreased (data not shown).

Figure 3 provides the results of experiments documenting the toxicity of LCFA on human Huh-7 hepatocytes. Survival rates were approximately 50% those of controls (no exposure to LCFA) following 24 hours of exposure to LCFA. However, the addition of the L-FABP inducers; clofibrate and simvastatin at concentrations of 5.5-100 µM had no concentration-dependent effect on cell survival following exposure to LCFA (Figures 4A and 4B respectively). Indeed, the only significant (p<0.05) finding in these experiments was a decrease rather than increase in hepatocyte survival following exposure to 12.5 µM of clofibrate.

Steroid Hormones and LCHAD expression

In time dependent experiments, B2325 cells (1×10^4 cells/well) were incubated with β-estradiol alone (50 nM), progesterone alone (550 nM) or a combination of β-estradiol (50 nM) and progesterone (550 nM) for 24, 48 or 72 hours. In concentration dependent experiments, B2325 cells were exposed to β-estradiol at concentrations of 5-500 nM for 72 hours. The concentrations of β-estradiol and progesterone approximated those reported in the serum of women in their third trimester of pregnancy.

Following 24, 48 and 72 hours of exposure to steroid hormones, B2325 cell mRNA was extracted by TRIZOL for LCHAD mRNA expression by RT-PCR and LCHAD protein by Western blot analysis as described by Spiekerkoetter et al.[]

Effect of L-FABP Inducers on Cell Survival

B2325 skin fibroblasts heterozygote for the E474Q mutation were kindly provided by Dr. R. Boriack at the Children’s Medical Centre, Dallas, TX and Huh-7 cells by Dr. Y. Gong at the University of Manitoba, Winnipeg, Canada. Polyclonal rabbit IgG LCHAD antibody was purchased from Protein-Tech Group, Inc. (Chicago, IL). β-estradiol, progesterone, clofibrate, simvastatin, oleic acid and palmitic acid were purchased from Sigma-Aldrich (St. Louis, MO) and WST-1 reagent from Roche Diagnostics (Indianapolis, IN).

MATERIALS AND METHODS

The low level of expression with β-estradiol at 24 hours was not sustained thereof, were the results of Western blotting for LCHAD protein expression by RT-PCR and LCHAD protein by Western blot analysis as described by Spiekerkoetter et al.[]

Figure 1 Effect of β-estradiol (50 nM), progesterone (550 nM) and a combination of both agents on long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) mRNA expression by RT-PCR in LCHAD deficient B2325 human fibroblasts after 24, 48 and 72 hours of exposure. The low level of expression with β-estradiol at 24 hours was not sustained nor evident when the combination of β-estradiol and progesterone were employed.
Previous studies have demonstrated that steroid hormones and simvastatin do not attenuate LCFA-induced hepatocyte injury. Liver steroid hormones do not alter the expression of LCHAD, a key functional enzymatic activity was not ascertained on LCHAD mRNA and protein expression were documented but functional enzymatic activity was not ascertained. Finally, both clofibrate and simvastatin were added to Huh-7 cells 24 hours after exposure to LCFA. Thus, the possibility remains that these agents could provide protection against the development of AFLP and/or HELLP if administered prior to the onset of injury. However, the majority of such cases appear after the clinical onset of the condition and recurrent disease with subsequent pregnancies is relatively uncommon.

In conclusion, the results of this study argue against steroid hormone induced suppression of LCHAD expression as contributing to the pathogenesis of AFLP and HELLP in pregnant women. The results also argue against upregulation of L-FABP as a therapeutic strategy for these conditions.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

1 Schutt VA, Minuk GY. Liver diseases unique to pregnancy. Best Pract Res Clin Gastroenterol 2007; 21(5): 771-792

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3 Treem WR, Shoup ME, Hale DE, Bennett MJ, Rinaldo P, Millington DS, Stanley CA, Riely CA, Hyams JS. Acute fatty liver of pregnancy, hemolysis, elevated liver enzymes, and low platelets syndrome, and long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Gastroenterol* 1996 Nov; 91(11): 2293-300.

4 Browning MF, Levy HL, Wilkins-Haug LE, Larson C, Shih VE. Fetal fatty acid oxidation defects and maternal liver disease in pregnancy. *Obstet Gynecol* 2006 Jan; 107(1): 115-120.

5 Strauss AW, Bennett MJ, Rinaldo P, Sims HF, O'Brien LK, Zhao Y, Gibson B, Ibda J. Inherited long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and a fetal-maternal interaction cause maternal liver disease and other pregnancy complications. *Semin Perinatol* 1999 Apr; 23(2): 100-112.

6 Haglind CB, Stenlid MH, Ask S, Alm J, Nemeth A, Doben U, Nordenstrom A. Growth in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency. *JIMD Rep* 2013; 8: 81-90.

7 Wojtczak L and Schonfeld P. Effect of fatty acids on energy coupling processes in mitochondria. *Biochim Biophys Acta* 1993; 1183: 41-57.

8 Kramer JH and Weglicki WB. Inhibition of sarcolemmal Na+-K+ATPase by palmitoyl carnitine: Potentiation by propranolol. *Am J Physiol* 2003 Oct-Dec; 285(4): H75-H81.

9 Mak IT, Kramer JH and Weglicki WB. Potentiation of free radical-induced lipid peroxidative injury to sarcolemmal membranes by lipid amphiphiles. *J Pharm Pharmacol* 2004; 56(9): 1155-1157.

10 Ibda JA. Acute fatty liver of pregnancy: an update on pathogenesis and clinical implications. *World J Gastroenterol* 2006; 12(46): 7397-7404.

11 Egerman RS, Sibai BM. HELLP syndrome. *Clin Obstet Gynecol* 1999; 42: 381-389.

12 Rajaraman G and Burczynski FJ. Effect of dexamethasone, 2-bromopalmitate and clofibrate on L-FABP mediated hepatoma proliferation. *J Pharm Pharmacol* 2004; 56(9): 1155-1161.

13 Rajaraman G, Wang GQ, Yan J, Jiang P, Gong Y and Burczynski FJ. Role of cytosolic liver fatty acid binding protein in hepatocellular oxidative stress: effect of dexamethasone and clofibrate treatment. *Mol Cell Biochem* 2007; 295(1-2): 27-34.

14 Seo M, Inoue I, Ikeda M, Nakano T, Takahashi S, Katayama S, Komoda T. Statins Activate Human PPARalpha Promoter and Increase PPARalpha mRNA Expression and Activation of HepG2 Cells. *PPAR Res* 2008; 2008: 316306.

15 Boroditsky RS, Reyes FI, Winter JS, Fainman C. Maternal serum estrogen and progesterone concentrations preceding normal labor. *Obstet Gynecol* 1978 Jun; 51(6): 686-691.

16 Spiekerkoetter U, Sun B, Khuchua Z, Bennett MJ, Strauss AW. Molecular and phenotypic heterogeneity in mitochondrial trifunctional protein deficiency due to beta-subunit mutations. *Hum Mutat* 2003 Jun; 21(6): 598-607.

17 Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, Brooke JC, McLaren DS, Channer KS, Jones TH. Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterole-fed androgen deficient mice. *Life Sci* 2014 Jul 30; 109(2): 95-103.

18 Cardoso CM, Moreno AJ, Almeida LM, Custodio JB. Comparison of the changes in adenine nucleotides of rat liver mitochondria induced by tamoxifen and 4-hydroxytamoxifen. *Toxicol In Vitro* 2003 Oct-Dec; 17(5-6): 663-670.

19 Bai X, Lin X, Drayton J, Liu Y, Ji C, Odle J. Clofibrate increases long-chain fatty acid oxidation by neonatal pigs. *J Nutr* 2014 Nov; 144(11): 1688-1693.

20 Yan J, Gong Y, Wang G, Gong Y, Burczynski FJ. Regulation of liver fatty acid binding protein expression by clofibrate in hepatoma cells. *Biochem Cell Biol* 2010 Dec; 88(6): 957-967.

21 Landrier JF, Thomas C, Grober J, Duez H, Percevault F, Souidi M, Linard C, Staels B, Besnard P. Statin induction of liver fatty acid-binding protein expression by clofibrate in androgen deficient mice. *FEBS J* 2003 Jun; 279(44): 45512-45518.

22 Luxon BA, Milliano MT, Weisiger RA. Induction of hepatic cytosolic fatty acid binding protein with clofibrate accelerates both membrane and cytoplasmic transport of palmitate. *Biochim Biophys Acta* 2000 Sep; 1487(2-3): 309-318.

23 Baqc Y, Assor P, Gendrot C, Perrotin F, Scotto B, Andres C. Recurrent acute fatty liver of pregnancy. *Gastroenterol Clin Biol* 2007 Dec; 31(12): 1135-1138.

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