HEPATIC AND PLACENTAL ENDOCANNABINOID SYSTEM (ECS) IN MATERNAL OBESITY

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Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the most prevalent form of liver pathology in the Western world and has been linked to obesity, insulin resistance, and type 2 diabetes with the incidence as high as 75-92% in the morbidly obese population. In general pediatric population the prevalence of NAFLD has been reported to be in the 13-14% range. Fatty liver has already been documented in fetuses of obese women and in healthy pregnant women as early as the first trimester of pregnancy. Recent data showed a direct role of endocannabinoids (ECBs) in alcoholic and non-alcoholic (obesity-related) fatty liver diseases. In particular, the activation of endocannabinoid receptor 1 (CB1) has been associated with hepatic fat accumulation in the animals on the higher fat diet. However, the role of CB1 in maternal and fetal responses to the obesity (Figure 1) has not been yet elucidated. Our goal was to use the baboon model of maternal obesity to evaluate the expression of CB1 receptors in maternal and fetal livers and to compare the expression between the groups.

Objectives

The goal of this study was to evaluate the expression of the ECB receptor CB1 within the liver of maternal obese and non-obese baboons (*Papio spp*), as well as their fetuses, to determine whether or not maternal ECB expression can influence CB1 hepatic expression in offspring used as an indicator of the risk for developing NAFLD.

Methods

Archived liver and placental tissues from a previous study in which samples were collected from obese and non-obese baboons (*Papio spp*) (Farley et al., 2009), were evaluated using Reverse Transcription real-time quantitative PCR method (Q-RT-PCR) using the Light Cycler 96 from Roche. The TRIzol method was used to isolate RNA from tissue samples (Life Technologies, USA), and cDNA was synthesized according to the manufacturer's instructions (Applied Biosystems/ Roche, USA). Q-RT-PCR was performed using Fast start Essential DNA Probe Master Mix (Roche, USA), and TaqMan Gene Expression Assay Probes (Life Technology, USA). The TaqMan probes used were CB1 (Hs01038522). A secondary analysis of the previously published data was also performed. IHC was done using the CB1 monoclonal primary antibody (Immunogenes; Budakeszi, Hungary), and the secondary antibody was included in the Vectastain ABC kit (Vector laboratories; Burlingame, CA); methodology for IHC was performed according to kit instructions. IHC slides were scanned using the NanoZoomer SQ and quantification was done using Aperio software.

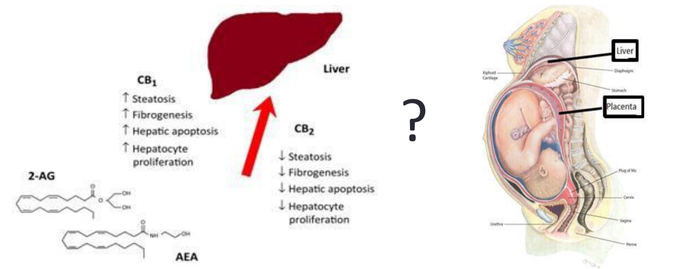

Figure 1. The endocannabinoid (ECB) system (ECS) of the liver. N-arachidonoylethanolamine (anandamide, AEA), and 2-arachidonoylglycerol (2-AG) are synthesized in the gut and liver, acting locally and in the brain. In the liver, the type 1 cannabinoid receptor (CB1) and CB2 have opposing effects, with CB1 promoting steatosis, fibrogenesis, apoptosis, and proliferation and CB2 inhibiting these effects (modified from Maccarrone et al., 2015).

Results

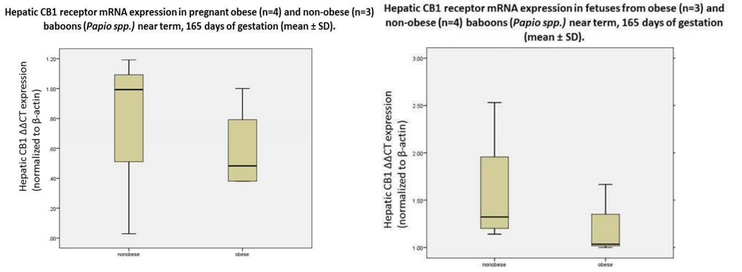

Figure 2. Hepatic mRNA CB1 Expression. Left panel: Maternal hepatic expression of CB1 in pregnant obese (n=4) and non-obese (n=3) baboons(p=0.9, Mann-Whitney test). Right panel: Hepatic expression of CB1 in fetuses born tomaternal obese (n=3) and non-obese (n=4) individuals (p=0.9, Mann-Whitney test).

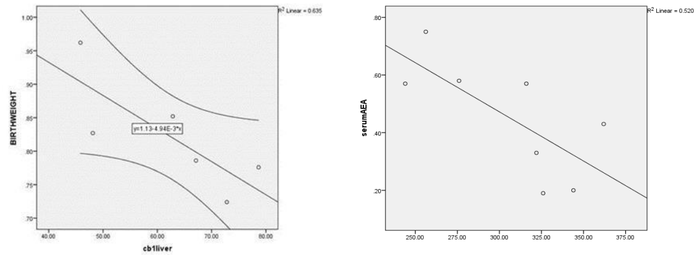

Figure 3. Correlation of CB1 receptor mRNA expression with fetal birthweight (left panel) and correlation of maternal liver weight with serum AEA concentration serum (right panel).

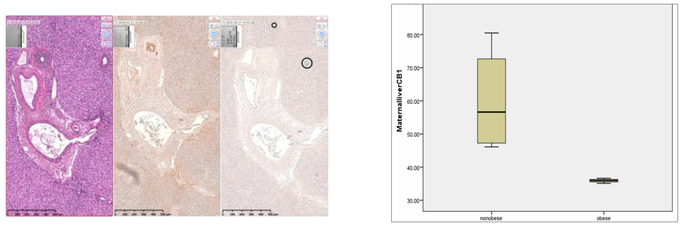

Figure 4. Representative image of CB1 IHC staining in hepatic lobule, it appears to stain membrane (and perhaps less so cytosolic) areas of hepatocytes, and is more prominent in centro-lobular hepatocytes. (H&E staining (left), positive CB1 antibody staining (middle) and negative control for CB1(right)). Hepatic protein expression of maternal CB1 receptor in obese (n=3) and non-obese (n=4) baboons (left) (p=0.57, Mann-Whitney test) (right).

Results Cont.

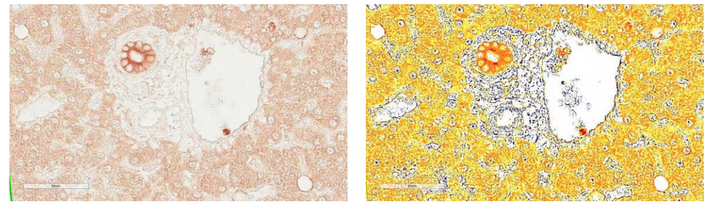

Figure 5. Representative image of CB1 expression in hepatic lobule (left). Right panel shows quantification algorithm applied in Aperio software: the positive (orange), weak positive (yellow), strong positive (red) and negative (blue) signal.

Conclusion

This is the first report demonstrating the presence of ECB receptors in fetal and maternal hepatic tissues. Hepatic CB1 protein expression was decreased in pregnant obese mothers. Fetal CB1 mRNA expression correlated with fetal birthweight. A negative correlation is demonstrated between serum AEA and maternal liver weight, pointing out possible involvement of the ECB pathway in fetal growth and maternal metabolic adaptations to pregnancy. Changes in the ECBs might be the candidates for being markers of NAFLD in pregnancy.

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