CANNABINOID-RECEPTORS MEDIATED REGULATION OF LONG-CHAIN POLY-UNSATURATED FATTY ACID (LC-PUFA) TRANSPORTER (MSFD2A) IN PLACENTAL AND BLOOD-BRAIN (BBB) BARRIERS.

Al-Ahmad Abraham[1], Samuel David[2], Maira Carrillo[3], Jay English[2], Stacy Martinez[3], Gary Ventolini[3], and Natalia Schlabritz-Lutsevich[3].

[1]School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX.

[2] University of Texas at the Permian Basin, Odessa, TX, USA.

[3]Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center at the Permian Basin, Odessa, TX, USA.

INTRODUCTION

Legalization of recreational marijuana raised issues regarding effects of exogenous cannabis in pregnancy. The cannabinoids family is comprised of 66 chemical products with the common structure of *Cannabis sativa* (Δ9Tetrahydrocannabinoid-THC), which binds to CB1R and CB2R main cannabinoid receptors. Exogenous cannabinoids are working through the mechanism of "kick-starting" the endogenous cannabinoid system (ECS). ECS are derivative of LC-PUFA. The LC-PUFA transporter- MSFD2A - is essential for the BBB integrity and placental syncytialization. Both receptors (CB1R and MSFD2A) are expressed in placenta and fetal brain, however, their role in the regulation of brain and placenta functions remains to be elucidated.

OBJECTIVE

The aim of this study was to evaluate whether CB1R and CB2R are involved in the regulation of MSFD2A transporter expression in placenta and brain models.

MATERIALS AND METHODS

***IN VITRO* MODEL OF HUMAN BBB, BASED ON INDUCED PLURIPOTENT STEM CELLS (IPSCS).**

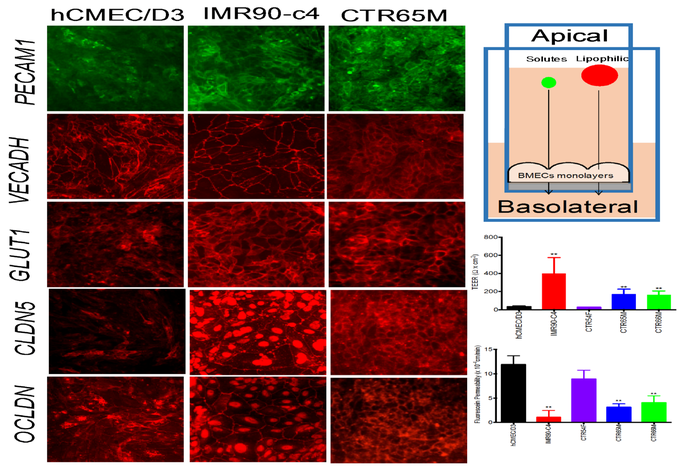


Figure Stem cell-derived model of the BBB. *Left panel:* Representative immunocytochemistry profile of iPSC-derived BMECs from two iPSC lines (IMR90-c4 and CTR65M). Note the paucity of tight junctions complexes in hCMEC/D3 cells. *Right panel:* The Transwell system. BMECs are seeded on top of a polyester membrane and allowed to form tight monolayers. n=4-5, *P<0.05 versus hCMEC/D3 group.

MATERIAL AND METHODS

It is important to demonstrate the expression and functional activity of an eCS at the fetal BBB. To achieve such goal, we used an in vitro model the human BBB based on induced pluripotent stem cells (iPSCs) developed by Shusta and colleagues (PMID: 24561821).

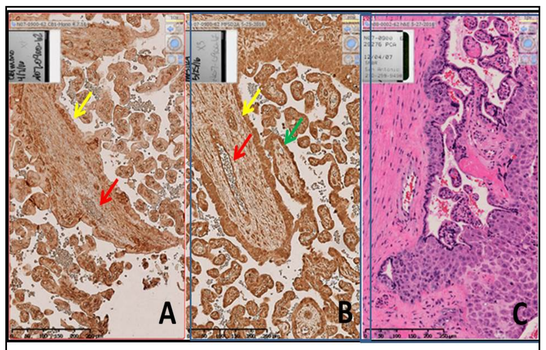
In this model, iPSCs are differentiated using a defined protocol that allows us to obtain functional BMECs monolayers after 10 days of differentiation . These cells were expressing key markers of the BBB, including tight junction complexes (occludin, claudin-5) capable to form tight monolayers (represented by a high transendothelial electrical resistance (TEER) and a low paracellular permeability to sodium fluorescein). These monolayers were carrying barrier properties similar or better than hCMEC/D3, a common adult immortalized cell line. In used BMECs differentiated from IMR90-c4 and CTR65M iPSC lines.

Human umbilical vein endothelial cells (HUVEC).

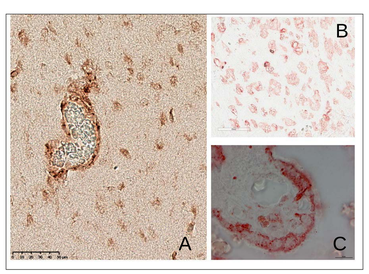
Cells were exposed to CB1R antagonist (AM125) and CB2R agonist (JWG 15) MSFD2a expression was quantified using Q-RT-PCR with the commercially available primer sets.

Immunohistochemistry : Commercially available antibodies were applied according to the previously published protocol (Gandhi et al., 2017) in human and baboons' placental tissue and banked baboon (*Papio spp*.) cerebral

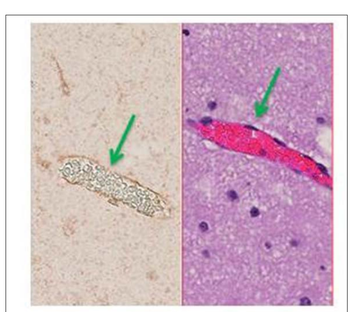
RESULTS



Representative images of the expression of CB1R (A) and MFSD2A (B) in baboon placenta near term. Notice expression of CB1R and MFSD2A in the cytotrophoblast (yellow arrow) and fetal endothelium (red arrow) and MFSD2A in the syncytiotrophoblast (green arrow). C – Hematoxylin and eosin staining (magnification 10X).

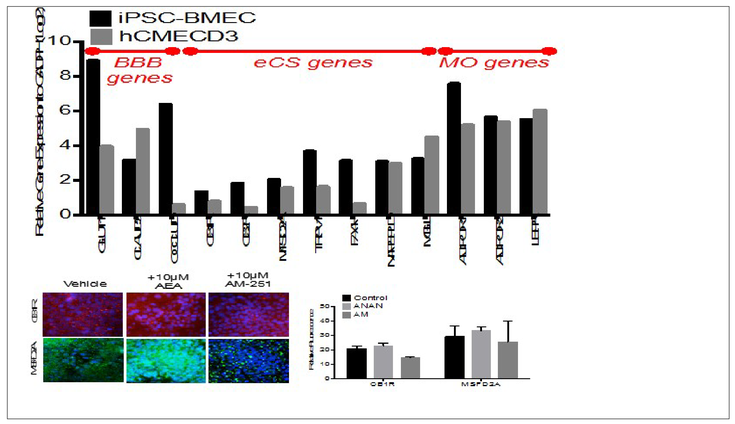


Expression of CB2R in the fetal endothelium and glial cells of the baboon fetal cortex at term (A) and in extra villous (B) and villous trophoblast, macrophages of human placenta (C,10.1016/j.placenta.2013.08.007).

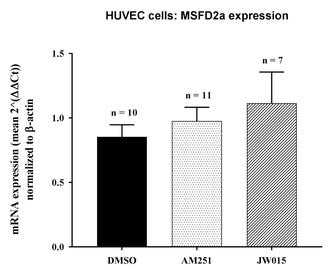


Expression of MSFD2A in fetal endothelial cells (cerebral cortex of the fetal baboon near term (165 dGA)), magnification 10X

DISCUSSION



eCS pathway at the blood-brain barrier (BBB). *Top panel:* Gene expression profile of key eCS and MO genes in IMR90-derived BMECs and hCMEC/D3 cells. Data are from in house experiments (Ipsc) and from existing literature (hCMEC/D3). Note the similar expression profile between the two cell lines. *Bottom panel:* Immunocytochemistry profile of MSFD2A and CB1R in IMR90-derived BMEC monolayers and relative fluorescence intensity. Note the slight increase following AEA treatment (CB1/CB2 receptor agonist) and decrease following AM-251 (CB1R antagonist).



MSFD2a mRNA expression in HUVEC cells, treated with CB1R antagonist (AM251) and CB2R agonist (JW015). Slight differences between treatment groups.

CONCLUSIONS

We demonstrated CB1R-dependent regulation of LC-PUFA transporter (MSFD2A) in BBB and CB2R-dependent regulation in the placental barrier.

ACKNOWLEDGEMENT

The authors wish to acknowledge the contribution of the TTUHSC Clinical Research Institute for their assistance with this research. We would like to acknowledge support of the Labor and Delivery personnel and residents/faculty of the OB&GYN Department. Authors would like to thank Dr. Zavada (Dean of art and sciences at UTPB) for the support.