A new in vitro model of BBB and brain organoids to study the role of Mg in brain development

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Human brain organoids (ORGs) are a widely used *in vitro* model that contribute to our knowledge about the biology and pathophysiology of nervous cells. They are 3D multicellular clusters that mimic the cytoarchitecture and the developmental pathways that occur *in vivo*. The most debated limitation of ORGs is the lack of the endothelial component. Thus, we have developed a co-culture *in vitro* system composed by the Blood Brain Barrier (BBB) and ORGs. To evaluate its strength, the cortical organization of the ORGs was assessed. In addition, the role of magnesium (Mg) supplementation was investigated with a focus on cortical neurons migration and neurodevelopment. The modulation of BDNF, a crucial neurotrophic factor involved in neurodevelopment, neuroplasticity and neurosurvival, was observed.

ORGs are generated from iPSCs with different differentiation media for 36 days. The BBB is composed by a co-culture of human brain endothelial cells and astrocytes in a transwell system. An inorganic and an organic Mg salt (Mg sulphate and Mg pidolate, respectively) were added to the culture media to reach the extracellular concentrations of 1 or 5 mM. The cortical layer organization was observed by immunofluorescence using CTIP2, TBR2 and SOX2 antibodies to detect the cortical, the subcortical neurons and the neural progenitor cells, respectively. Ultrastructural analysis was performed using transmission electron microscopy. Intra-ORGs and released BDNF were detected by qRT-PCR and ELISA.

Our data demonstrate that BDNF levels are higher in the new in vitro model because of the presence of the endothelial component, which is the main responsible for its secretion. Moreover, the cortical layer is more organized in the presence of the BBB. In addition, high Mg salts concentration ameliorates the organization of the ORGs cultured in the presence of the BBB. This data is explained by the increased release of endothelial BDNF when the BBB is treated with high Mg salts, which results in the upregulation of intra-ORGs BDNF levels.

This study underlines the need of having a comprehensive in vitro model to study the CNS, given the importance of the crosstalk between BBB and brain in both physiological and pathological conditions. Moreover, it unveils the role of Mg in brain development and in modulating the release of BDNF from the BBB.