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

Giorgia Fedele[1], Marco Bartolini[1], Isabella Zafferri[1], Sara Castiglioni[1] and Alessandra Cazzaniga[1]

[1] Department of Biomedical and Clinical Sciences, Università di Milano, 20157 Milano, Italy

OVERVIEW

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 occurring in vivo. The most debated limitation of ORGs is the lack of vascularization. Thus, we have developed a co-culture in vitro system composed by the Blood Brain Barrier (BBB) and ORGs.

INTRODUCTION

The BBB is actively involved in the exchange of substances between the blood and the brain. BBB dysruption has been observed in many neurological and mental disorderds, underlyining the importance of a functional BBB, and of having a comprehensive in vitro model. The purpose of the study was to generate a simple but comprehensive model of BBB-ORGs, and to exploit it to analyze the effects of Mg supplementation on human brain development, since the mechanisms of Mg impact on brain are unknown.

METHODS

ORGs are generated from iPSCs. The BBB is composed by a co-culture of HBMEC and HA in a transwell system. An inorganic and an organic Mg salt (Mg sulphate and Mg pidolate) 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, Intra-ORGs and released BDNF were detected by qRT-PCR and ELISA.

RESULTS

BDNF levels are higher in the BBB-ORGs 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.

CONCLUSIONS

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. Further analyses are ongoing to better assess the mechanisms behind these findings, with a focus on other neurotrophic factors and on Mg transporters.