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Interaction of enterovirus A71 with the human blood-brain barrier: an in vitro study

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INTRODUCTION
Enterovirus A71 (EV-A71) is an RNA non-polio enterovirus that belongs to the Picornaviridae family. This virus is implicated in epidemic disease of hand-foot-mouth disease (HFMD) mostly in infants and children, especially in Asian countries. In Europe since 2015, the emergence of a multi-recombinant C1-like EV-A71 (C1v2015) induced outbreak of meningitis and brainstem encephalitis in Spain (2016) and France (2016, 2018). Damage to the blood-brain barrier (BBB) could explain such increase in the severity of symptoms.

The BBB is the larger interface between blood and brain, very selective, with a microvasculature of 600 km. Endothelial cells (ECs) constitute the wall of brain capillaries and confer to the barrier its function by expression of many adherent and tight junction proteins (ZO-1, claudin-5), specific transporters (Glut-1) and efflux pumps (P-gp, BCRP). Pericytes, astrocytes end feet and neurons, which compose the neurovascular unit, surrounds ECs and influences them.

This work aimed to investigate the passage of free EV-A71 strain through a multi-cellular in vitro model of BBB.

RESULTS

Paracellular Permeability
Basal fluorescence was measured after incubation (1h) with a hydrophilic compound : Lucifer Yellow (LY).

Trans-Endothelial Electrical Resistance (TEER)
The higher the measured resistance, the stronger the tight junction.

Immunofluorescence (IF)
Detection of tight junction protein zonula-occludens 1 (ZO-1).

Functional test : presence of efflux pump P-gp
Comparison of intracellular fluorescence of Rhodamine 123 which naturally enter into EC, release by the efflux pumps P-gp, in the presence or not of a pump inhibitor (Elacridar).

CD34+ derived endothelial cells
Cultivated on the apical side of Transwell™ and coating with Matrigel™

Bovine pericyte
Cultivated on the basal side of Transwell™ and coating with type I-collagen

Percentage of infected cells realized from 10 confocal microscopy images.

Fig. 1 Percentage of infected cells at 24h pi

Fig. 2 Endothelial paracellular permeability after 24h and 72h post-infection

An increase of permeability is observed 24h pi between the positive control and negative control. No difference is observed between the negative control and EV-A71 infections.

Fig. 4 Endothelial paracellular permeability (A) and viral titration (B) in an inflammatory environment with/without C1/16 infection

A. With luminal TNF-α

B. C1/16 infection

The TNF-a treatment increases the permeability at 24h with or without infection (Fig 4.A). The infectious virus is quantifiable only in the luminal side (below 40,000 infectious particles) (Fig 4.B).

CONCLUSION
The integrity of the BBB-like model generated in vitro by a co-culture of CD34+ derived ECs and pericytes displayed limited alteration after EV-A71 infection. Results provide evidence of a low viral replication in ECs and limited crossing through the in vitro BBB model even in an inflammatory environment. Future investigations will focus on the transmigration of EV-A71 through infected leukocytes.

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