Astrocytes – endothelial interaction influences β-amyloid effects at the blood brain barrier

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The blood brain barrier (BBB) that consists of specialized endothelial cells, pericytes and astrocytes, is able to modulate the access of small molecules, drugs and immune cells towards the brain. The barrier is often involved in neurological affection and lately alterations in its function have been described in Alzheimer's disease (AD). The deposition of beta amyloid (Aβ) and neuronal degeneration can be anticipated by alterations in BBB properties. It is well known that Aβ challenge can modify astrocyte activities and that Aβ accumulation can affect vascular functions. Due to their strict interaction, modifications in astrocytes' features influence endothelial properties at the BBB. Here, using an *in vitro* BBB model, we investigated the effects of $A\beta_{1-42}$ on barrier properties by testing the permeability to FITC-conjugated dextran. Further, to assess endothelial monolayer's integrity, the expression of claudin-5 and occludin, the principal constituents of the tight junctions (TJ), was evaluated. Human endothelial cells were cultured *in vitro* in a monolayer or co-cultured with astrocytes. When directly exposed to 2 μM $A\beta_{1-42}$ for 18 hours, endothelial cells' properties were not modified. When endothelial cells were co-cultured with astrocytes, Aβ treatment increased their permeability and reduced the expression of claudin-5. Reduced claudin-5 expression was associated to enhanced expression and activity of metalloproteinase 9 (MMP9), known to mediate the disruption of tight junctions. These events were not observed when endothelial cells were directly exposed to Aβ. To assess whether astrocytes released factors able to modulate Aβ effects on endothelial cells, astrocytes were exposed to 2 μM $A\beta_{1-42}$ and conditioned medium (ACM) was collected after 18 hours. Endothelial monolayer was then exposed to 2 μM $A\beta_{1-42}$ in the presence of ACM from astrocytes previously challenged with Aβ. In the presence of ACM, $A\beta_{1-42}$ treatment increased FITC-conjugated dextran permeability and reduced claudin-5 expression.
Among factors released by astrocytes that could modulate BBB properties, we focused our attention on the role that vascular endothelial growth factor (VEGF) may play in this model. VEGF modulates several vascular functions and its overexpression in pathological conditions, such as ischemia, has been associated to BBB breakdown. Astrocytes were thus exposed to $A\beta_{1-42}$ and, in these conditions, an increased expression of both VEGF RNA messenger and protein was observed. To further confirm the involvement of VEGF in the effects on barrier properties after ACM exposure, endothelial monolayer was exposed directly to VEGF (100 ng/ml). Barrier properties were affected and the expression of MMP9 was increased, mimicking the effects observed after ACM exposure. Identifying the factors and pathways able to modify endothelial cells sensitivity to Aβ can be a useful step to prevent BBB modifications, eventually reducing the infiltration of molecules and immune cells that can precipitate neuronal degeneration.