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A bovine in vitro blood-brain barrier model under oxygen-glucose deprivation (OGD) condition

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Aim
During stroke, the brain endothelium experiences low glucose and oxygen. We therefore wish to investigate the effects of low glucose and oxygen in cultured brain capillary endothelial cells, focusing particularly on barrier properties and transport proteins.

Abbreviations
BBB: Blood-brain barrier; OGD: Oxygen-glucose deprivation; PCR: Polymerase chain reaction; RT: reperfusion; TEER: Transendothelial electrical resistance.

Background
Ischemia is a devastating disease which affects million of people every year. During ischemia, the loss of regional cerebral blood-flow and the subsequent reperfusion induce significant changes in the transport pathways and barrier properties of the blood-brain barrier (BBB).

Oxygen-glucose deprivation (OGD) protocol in a bovine blood-brain barrier in vitro model
Figure 2. Bovine brain endothelial cells were grown from capillary fragments in culture flasks for 5 days. They were then trypsinized and co-cultured with rat astrocytes in coated filter merts for additional 6 days. Thus, they were subjected to oxygen-glucose deprivation (OGD) conditions for 4 hours in a hyperoxygen waterbath and a subsequent "reperfusion" for 24-hrs.

Permeability increased during OGD and recovered during "reperfusion"

Conclusions
• The brain endothelial cells, co-cultured with astrocytes, showed a decrease in tightness during OGD, an effect which was reversible upon reperfusion.
• The tight junction proteins Claudin-5 and ZO-2 translocated from the junction complex to the cytosol during OGD, and relocated to junctions during reperfusion. Their protein levels decreased during OGD and recovered upon reperfusion.
• The transporter GLUT1 migrated to the cell border during reperfusion.
• Pgp protein expression decreased during reperfusion. The protein level of HBEFG, LRP1 and InsR increased in the reperfusion phase.

Future experiments
• Evaluating the degree of Pgp activity reduction after 24h of reperfusion by testing the permeation of known Pgp substrates across the endothelial cell monolayer.
• Investigating the possibility that the receptors HBEFG, LRP1 and InsR may mediate the delivery of drugs across the ischemic BBB by transport experiments.
• To examine other brain cell types influence on barrier properties and after the OGD treatment.

References
1. From S.M. Allan & N.J. Rothwell, 2001

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