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FACULTY OF HEALTH AND MEDICAL SCIENCES

A bovine i*n 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; R: reperfusion; TEER: Transendothelial electrical resistance.

Background

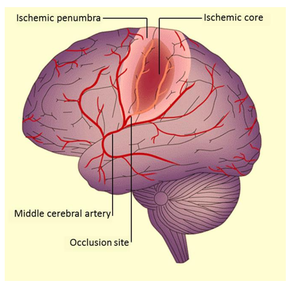


Figure 1. Brain affected by ischemic stroke.

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 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

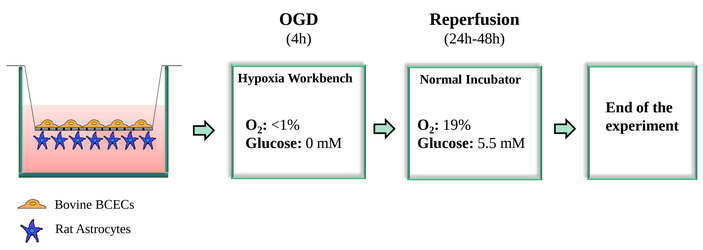
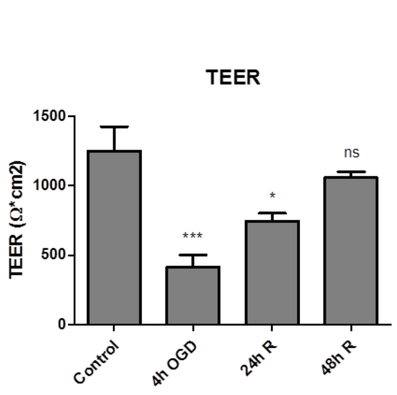
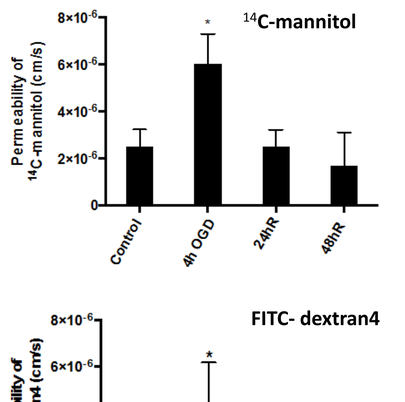


Figure 2. Bovine brain endothelial cells were grown from capillary fragments in culture flasks for 5 days. They were then trypzinized and co-cultured with rat astrocytes in coated filter inserts for additional 6 days. Thus, they were subjected to oxygen-glucose deprivation (OGD) conditions for 4 hours in a hypoxia workbench and a subsequent "reperfusion" for 24-48h.

Permeability increased during OGD and recovered during "reperfusion"





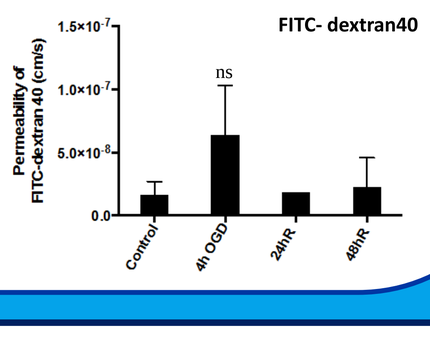


Figure 3. TEER was measured at various time points, before, under and after the OGD protocol. Measurements were performed with an Endohm cup electrode. N=3-6, n= 6 (left figure). Paracellular tightness was also evaluated by determining the passive permeability of 3 marker molecules: ^{14}C-mannitol, FITC-dextran4 and FITC-dextran40. N=3-4, n=3 (right figures)

CLDN-5 and ZO-1 translocated from the cell junctions during OGD. GLUT1 localized in the cell mebrane during reperfusion

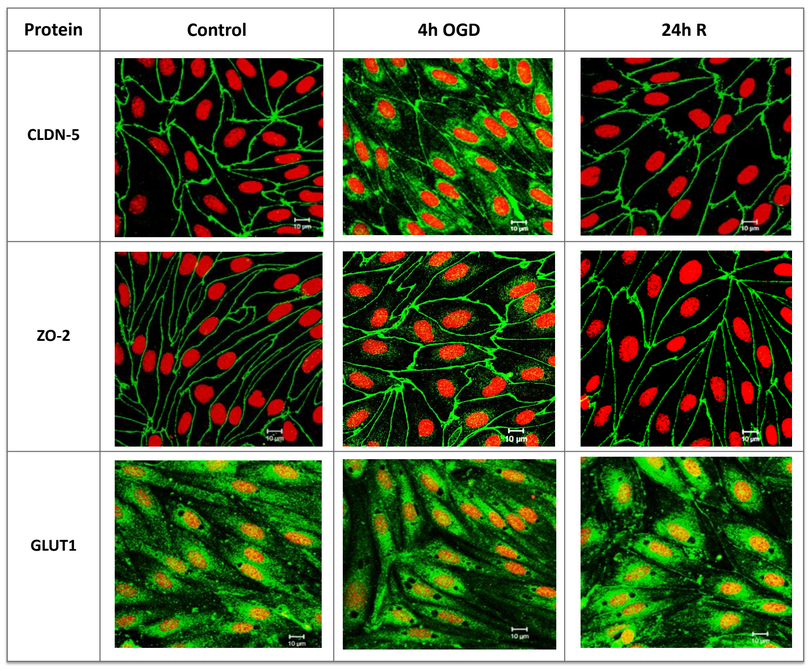


Figure 4. Confocal laser scanning microscopy images of brain endothelial cells co-cultured with rat astrocytes . Cells were fixed, permeabilized and immunostained with Claudin-5, ZO-2 or GLUT-1 antibody (green), cell nuclei were counterstained with propidium iodide (red). n=4.

mRNA and protein expression level of selected proteins before, during and after OGD

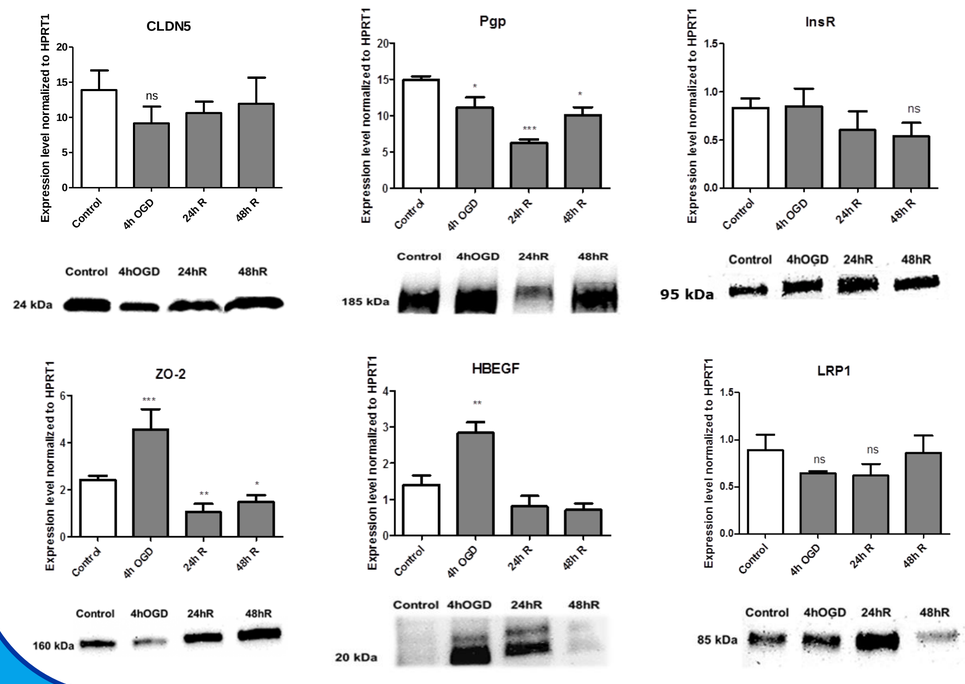


Figure 5. mRNA expression of target genes was investigated by RealTime-PCR using primers designed for bovine. n=4, (upper figures). Proteins were isolated from bovine endothelial cell/rat astrocyte in non contact co-cultures. An equal amount of proteins was loaded in each lane of the gel for western blot analisys. (n=2). (lower figures).

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 relocalized to junctions during reperfusion. Their protein level 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 HBEGF, 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 HBEGF, 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 during and after the OGD treatment.

References

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

Acknowledgement

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RIBBDD
Research Initiative on Brain Barriers and Drug Delivery