Cannabidiol restores the human inner Blood-Retinal Barrier after Oxygen-Glucose Deprivation in an in-vitro insulin-resistant model

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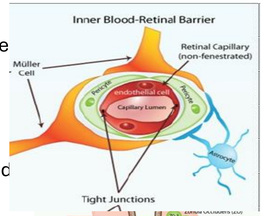
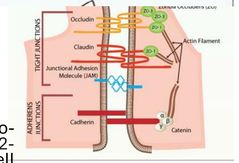
AIMS

1. Find optimal laboratory conditions for making an insulin-resistant model of the diabetic inner **Blood-Retinal Barrier** (iBRB).
2. Investigate the role of **Cannabidiol** (CBD) and the Peroxisome-Proliferator-Activated Receptor **gamma (PPARγ)** following a period of **Oxygen-Glucose Deprivation** (OGD).
3. Use **Trans-Endothelial Electric Resistance** (TEER) to measure iBRB competence.

BACKGROUND

1. Retinal capillary endothelial cells (REC) form the interface between systemic circulation and inner retina, together with pericytes and glia.
2. Tight junctions between adjacent REC borders regulate paracellular transport.
3. The iBRB may breakdown in diabetes causing oedema and vision loss.
4. Adjunctive therapy to steroids, immunotherapy and laser may benefit treatment outcomes.





ENDOCANNABINOID SYSTEM

The retina has synthetic and catabolic enzymes for the endo-cannabinoid system[1] with ligands Anandamide (AEA) and 2-arachidonylglycerol (2-AG) being synthesized on demand from cell membrane lipid precoursers.

Cannabinoid receptors (CB1, CB2, GPR55, TRPV1) are found on vascular, neuronal, glial and inflammatory cell-surface membranes and internally on mitochondria.

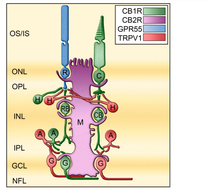
The Peroxisome-Proliferator-Activated Receptor Gamma[2] (PPARγ) is a Ligand-activated transcription factor. It forms heterodimers with RXR which bind to promotor regions.

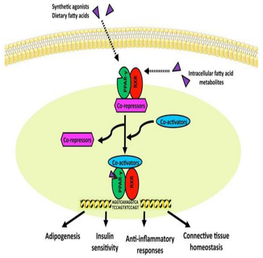
PPARγ activation modulates lipid and glucose metabolism and reduces pro-inflammatory mediators.

Endo-Cannabionid levels are elevated in diabetic aqueous humour[3]

Cannabidiol (CBD) is a non-psychoactive phyto-cannabinoid with neuro-protective and anti-inflammatory actions. It has a low affinity for CB1 and CB2 receptors but is an inverse agonist for GPR18 and GPR55, and is a PPARγ agonist.

CBD reduces VEGF production in STZ-induced diabetic rat retina





METHOD

A 3-CELL iBRB model (Hind WH et al) using human REC, astrocyte and pericytes at P4-5 (Innoprot) was developed for 24-Well plates with different combinations of co-cultures either side of 4µm pore PLL-coated inserts (Corning).

Cells were seeded at 7.5x10^4 cells/cm^2 (REC and Astrocytes) and pericytes at 2.5x10^4 cells/cm^2. in 1:1:1 astrocyte/ pericyte/ REC growth media and incubated at 37°C/ 5%CO_2 for 3 days to confluence.

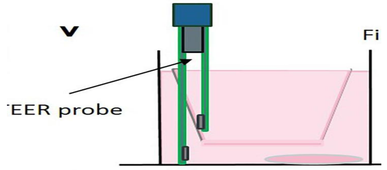
Cells were incubated for 18 hours in growth media with 20mM glucose and 200mM Palmitate conjugated to Fatty-acid free BSA (Sigma-Aldrich) to induce hyperglycaemia and Insulin resistance. Normal growth media was used as a control.

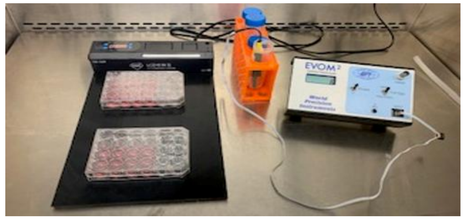
10µM Cannabidiol in ethanol vehicle was added to the apical surface of the wells with or without 10µM PPARγ inhibitor (GW9662, TOCRIS).

Oxygen-Glucose Deprivation was generated by changing media to glucose-free DMEM (Gibco) and placing the wells in a zip-loc bag with EZ GasPak system (BD) for anaerobic incubation for 4 hours at 37°C after which aerobic respiration with 5mM glucose was restored.

TEER (EVOM2 WPI) measurements taken before and following OGD using a heated mat at 37oC.

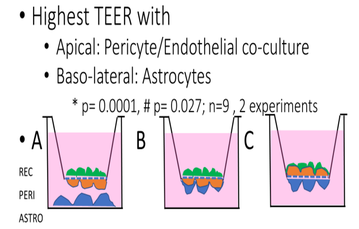
The mean TEER of 9 wells from 2 experiments was compared by ANOVA (GraphPad v 9.5.1) with Sidaks correction for multiple comparisons

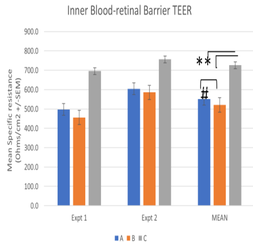




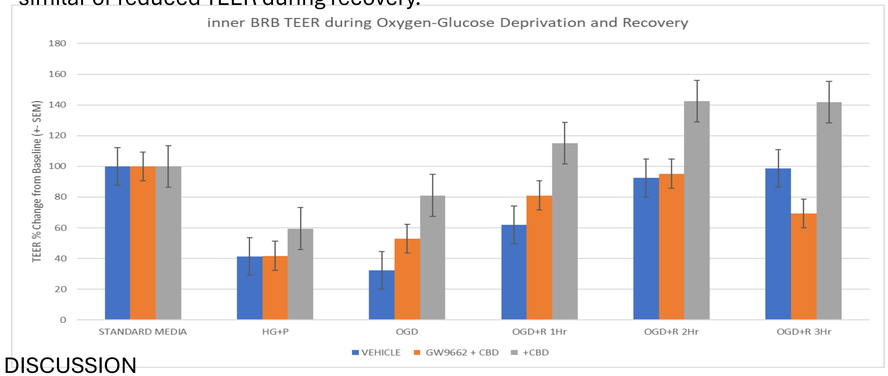
RESULTS

- Highest TEER with
  - Apical: Pericyte/Endothelial co-culture
  - Baso-lateral: Astrocytes





- 18 hour incubation in Palmitate/hyperglycaemia media significantly reduced TEER from baseline with further reductions after 4hours of OGD ($p<0.0001$)
- Cells with CBD had smaller reduction in TEER after OGD and during recovery the values were greater than baseline.
- Cells with CBD and GW9662 had greater TEER values than vehicle only following OGD but similar or reduced TEER during recovery.



DISCUSSION

We describe a 3-cell model of the iBRB with human retinal cells in which Apical Co-culture REC with Pericytes provides greater TEER.
Palmitate-Induced insulin-resistance in hyperglycaemia with an episode of oxygen-glucose deprivation more accurately reflects the diabetic micro-environment

CBD Reduces the impact of Oxygen-Deprivation and improves the recovery phase GW9662 partly inhibits CBD action indicating the role of PPARγ.

Ongoing studies are required to evaluate other ECS receptor pathways and to develop a topical delivery system to permit CBD to cross the cornea.