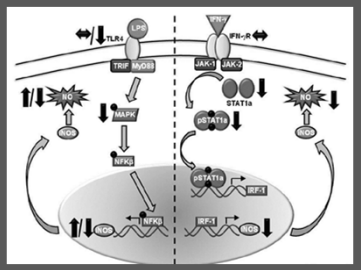
The Effect of Lipopolysaccharides (LPS) on Inducible Nitric Oxide Synthase (iNOS) in Human Microvascular Endothelial Cells (HMEC-1) and Human Choroidal Endothelial Cells (hCECs)

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Background

- TLR--A family of proteins initiating the innate immune response in reaction to invading microbes, involved in the pathogenesis of choroidal and retinal disease
- TLR4 proved to be expressed more in both HMEC-1 and hCECs
- LPS--Present in many Gram-negative bacteria and selected Gram-positive bacteria, recognized by TLR4
- iNOS--A family of enzymes catalysing the NO production, known angiogenesis mediator
- TLR4-LPS complex-MAPK and NF-kB pathways-iNOS expression



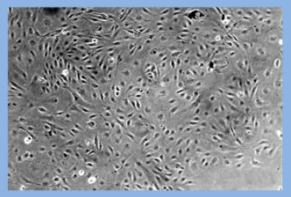
Aims

The purpose of this study was to investigate the expression of iNOS in endothelial cells following LPS stimulation.

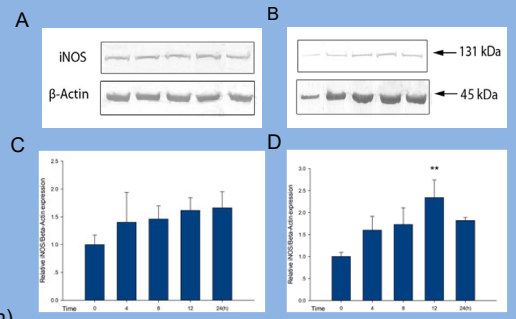
Materials and Methods

HMEC-1 were cultured on tissue-culture dishes. hCECs were isolated from 3 donors' eyes and cultured until they reached 70% confluency. LPS was then used to stimulate the cells in a 24-hour time course (0, 4, 8, 12 and 24h). The iNOS expression was then studied using Western blotting.

Results



Phase contrast photomicrograph of primary cultures of hCECs, demonstrating a typical cobblestone appearance (20x original magnification)



24-hour time course of iNOS protein expression in HMEC-1 and hCECs after LPS administration. Western blot analysis of iNOS in HMEC-1 (A, C) and hCECs (B, D) at time points indicated. Bands migrating at 131 and 45 kDa were detected using antibodies directed against iNOS and β-Actin, respectively. B: iNOS bands were quantitated and expressed as fold change values. Means±SEM are plotted. Images representative of 3 independent experiments are shown (n=3). Significance was determined using relative iNOS/Beta-Actin expression values by ANOVA with repeated measures. Significant differences were observed in hCECs from 12h time point. **p<0.01.

Conclusion

- iNOS expression up-regulated in time-dependent manner following LPS stimulation
- iNOS expression more prominent in primary cells
- iNOS playing a critical role in choroidal neovascularisation
- TLR4-LPS could be a mechanism for initiating angiogenic factors associating/ affecting progression of ocular neovascular diseases, e.g. AMD

References

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