Role of Vascular Endothelial Growth Factors on Ethanol-Induced Lymphatic Endothelial Cell Hyperpermeability

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Background

- In recent studies we found that treatment with ethanol increases lymphatic endothelial cell (LEC) permeability that is not associated with disruption of LEC tight junctions, adherens junctions or changes in JNK or ERK ½ expression
- Evidence has shown that lymphatic dysfunction is associated with PROX-1 deletion and Vascular Endothelial Growth Factor Receptor 3 (VEGFR3) inactivation
- Binding of vascular endothelial growth factor C (VEGF-C) and VEGF-D to VEGFR-3 regulates LEC differentiation and proliferation
- The homeobox transcription factor Prox1 is expressed in LECs. Prox1 and VEGFR-3 are coexpressed in LECs
- *Prox1*$^{+/-}$ mice with a defective lymphatic vasculature develop obesity caused by lymphatic hyperpermeability and leakage
- Overall, decreases in VEGFR3 and/or PROX-1 transcription in LECs will likely impact lymphatic function and permeability

Hypothesis

- ***We hypothesized that ethanol induces LEC leakage via disruption of Prox-1 and VEGFs pathways***

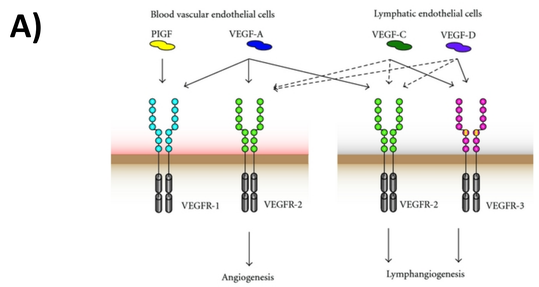

Figure 1: **A)** VEGF-C & VEGF-D are important regulators of lymphangiogenesis through interaction with VEGFR-3 in lympathic endothelial cells **B)** Cell culture before plating into 6-well plate for alcohol treatment

Methods

- Rat lymphatic endothelial cells were used to investigate VEGF-C, VEGF-D, PROX-1, and VEGFR-3 expression
- Cells were incubated with alcohol-supplemented cell culture media for 30 minutes, 7 and ½ hours (based on permeability studies)
- RNA was isolated
- Quantitative Polymerase Chain Reaction (qPCR) was used to measure expression of target genes; data were normalized against RPS and expressed as fold change

Alcohol (30m) ↓ PROX-1, VEGF-D, and VEGFR-3 gene expression

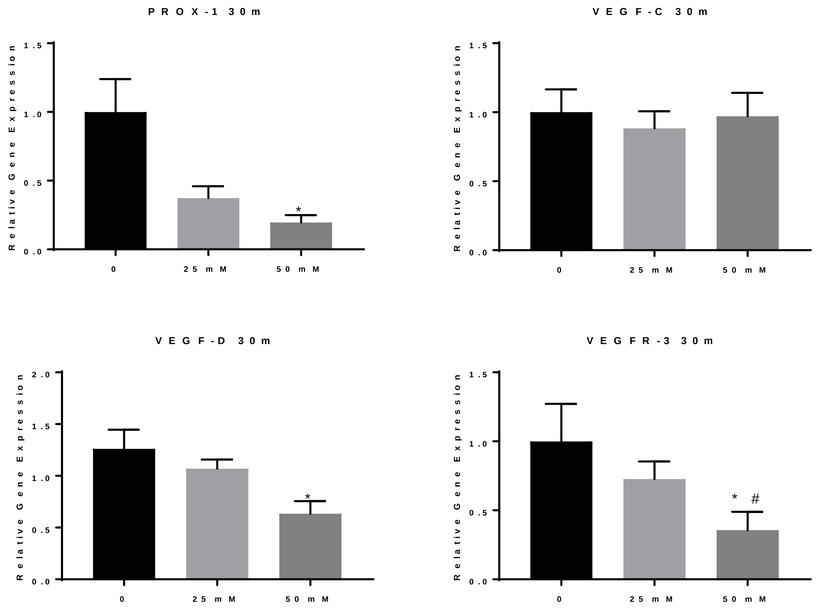

Figure 2: Relative gene expression of PROX-1 (top left), VEGF-C (top right), VEGF-D (bottom left), and VEGFR-3 (bottom right) in LECs after 30 minutes of ethanol (0, 25 – light gray or 50mM – dark gray) treatment. Ethanol at 50 mM concentration decreased VEGFR-3 and MLCK expression in LECs after 30 minutes. *p<0.05 *vs* 0mM; # p>0.05 *vs* 25mM

Alcohol (7.5h) ↓ VEGFR-3 gene expression

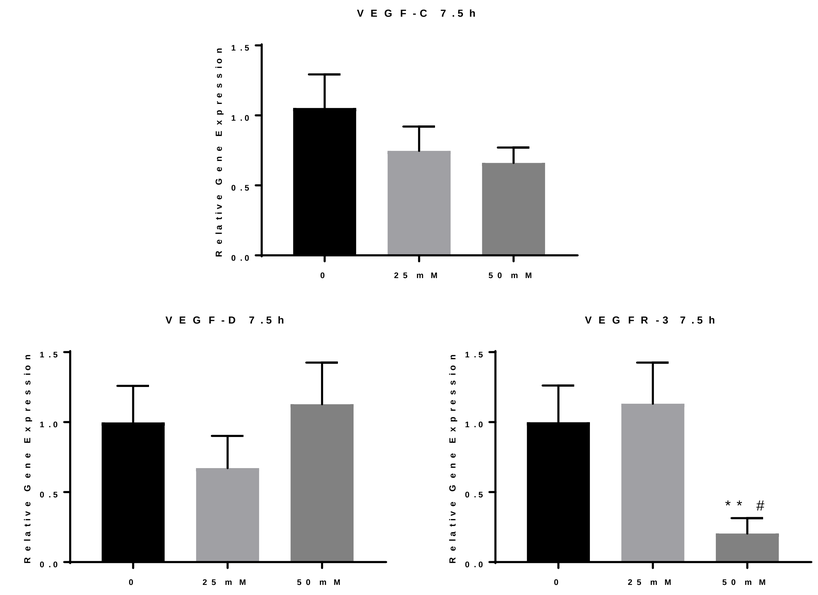

Figure 3: Relative gene expression of VEGF-C (top), VEGF-D (bottom left) and VEGFR-3 (bottom right) in LECs after 7.5h of ethanol (0, 25 – light gray or 50mM – dark gray) treatment. Ethanol at 25 and 50 mM concentration decreased VEGFR-3 expression in LECs after 7.5 hours. **p<0.01 vs 0mM; # p<0.05 vs 25mM

Conclusion

- Ethanol significantly decrease gene expression of PROX-1, VEGF-D, and VEGFR-3
- These data suggest that PROX-1, VEGF-D, and VEGFR-3 might be playing a role in alcohol-induced LEC permeability
- Future studies using specific inhibitors of VEGF-D and VEGFR-3 on LEC permeability will elucidate the role of these molecules on ethanol-induced LEC hyperpermeability

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