

P0026

Poster Session I

News from the fungal frontier

ACIDOSIS PROMOTES RHIZOPUS ORYZAE-INDUCED ENDOTHELIAL CELL INJURY IN VITRO VIA OVEREXPRESSION OF HOST GRP78 RECEPTOR AND FUNGAL COTH3

T. Gebremariam¹, J. Edwards Jr.¹, S.G. Filler¹, **A.S. Ibrahim¹**

¹Medicine, Los Angeles Biomedical Research Institute, Torrance CA, USA

Objectives: Mucormycosis (mainly caused by *Rhizopus oryzae*) is characterized by angioinvasion and vascular thrombosis. Previously, we showed that *R. oryzae* invades human umbilical vein endothelial cells (HUVECs) via binding of the fungal cell surface invasin Coth3p to host cell receptor, Glucose Regulated Protein 78 (GRP78). Since diabetics in ketoacidosis (DKA) are uniquely susceptible to mucormycosis, we sought to investigate the effect of physiological concentrations of beta-hydroxybutyrate (BHB) on the expression of Coth3p/GRP78 and subsequent *R. oryzae* ability to invade and damage HUVECS in vitro.

Methods: *R. oryzae*, HUVECs, or both were incubated with varying physiological concentrations of BHB (5-20 mM) prior to quantification of the expression of *CotH* or *GRP78* by qRT-PCR. *R. oryzae*-induced invasion (using Uvetix stain) and injury (by ⁵¹Cr-releasing assay) to HUVECs were also quantified after exposure of *R. oryzae*, HUVECs, or both to BHB. Statistical comparisons were done using the Wilcoxon Rank Sum test.

Results: Concentrations of 5 or 20 mM of BHB resulted in 3 and 9 fold increase in expression of HUVECs *GRP78* and 2 and 4 fold increase in *R. oryzae* *Coth3*, respectively ($P < 0.05$ when compared to cells without BHB, n=6 per group). Exposure of HUVECs to 9 mM BHB enhanced their *R. oryzae*-induced invasion and subsequent damage by ~ 100% ($p < 0.001$, n=6 per group). There was a less dramatic effect in invasion and damage of HUVECs after exposure of *R. oryzae* to BHB (~30% increase in both invasion and damage compared to cells that have not been exposed to BHB). Exposure of both *R. oryzae* and HUVECs to BHB did not significantly enhance *R. oryzae*-induced HUVECs invasion and damage over values seen with only HUVECs exposed to BHB. Finally, BHB had no effect on *R. oryzae* adherence to HUVECs.

Conclusion: Acidosis due to BHB enhances the expression of *GRP78* and *CotH3* expression. This enhanced expression results in increased ability of *R. oryzae* to invade and damage HUVECs in vitro. Additionally, it appears that invasion and damage to HUVECs are driven more by up-regulation of the receptor GRP78 than the Coth3 ligand. These results further explain the unique susceptibility of DKA patients to mucormycosis.