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Restoring the endothelial barrier function in the elderly

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**Abstract**

Endothelial barrier dysfunction in the elderly has been associated with severe disorders, including acute respiratory distress syndrome, sepsis and COVID-19. Herein we deliver an opinion regarding the development of alternative therapeutic avenues to counteract the pathogenesis of the corresponding diseases.

**Keywords:**

Inflammation
Acute lung injury
Acute respiratory distress syndrome

Endothelial barrier dysfunction is associated with Acute Lung Injury (ALI) and its more severe and lethal form, namely the Acute Respiratory Distress Syndrome (ARDS) (Barabutis et al., 2016). The current medical countermeasures to reduce the ARDS pathophysiology do not suffice, as demonstrated in the case of COVID-19. More than 500,000 people have already succumbed to the pandemic, with the elderly population being the majority of the deceased (Aw et al., 2020). Recent observations suggest novel ways to support the lungs of those subjected to SARS-Cov-2 related ARDS. The lung endothelium is a target of the COVID-19-related “cytokine storm”, causing lung endothelial permeability, edema and respiratory complications. Discovering pharmacological interventions to propel repairing processes in the affected endothelium, will most probably contribute in our battle against COVID-19, ARDS and sepsis.

Unfolded protein response (UPR) is a intracellular mechanism which includes the protein kinase RNA-like ER kinase (PERK), the activating transcription factor 6 (ATF6), and the inositol-requiring enzyme-1α (IRE1α) to propel repairing responses (Hetz et al., 2019). Hence, a targeted mild UPR activation may deliver promising therapeutic possibilities against ARDS (Barabutis, 2019). Upon robust endoplasmic reticulum (ER) – induced UPR activation, the cells will undergo apoptosis (Soltanmohammadi et al., 2021).

It was recently shown that the UPR suppressor Kifunensine weakens the endothelial barrier integrity of bovine pulmonary artery cells in a dose-dependent manner (Akhter et al., 2020a). Measurements of trans-endothelial resistance (indicator of barrier integrity) substantiated our findings. Furthermore, this α-mannosidases inhibitor (Kifunensine) affected key cytoskeletal modulators, since it induces the actin-severing activity of coflin, and enhanced the expression of the filamentous actin stress fibers. Observations suggested that UPR is involved in the maintenance of the endothelial barrier integrity (Kubra et al., 2020a).

Lipopolysaccharides (LPS) causes lung endothelial barrier disruption via Toll-like receptor (TLR)4 activation, and causes in vivo ALI/ARDS (Lu et al., 2008; Chan et al., 2019). TLR4 suppresses the ER stress marker C/EBP Homologous Protein (CHOP) (Hu et al., 2018; Bagratuni et al., 2019; Nishitoh, 2012), and LPS pre-treatment of mice subjected to ER stress results to similar effects (CHOP reduction) (Woo et al., 2009). Hence, this endotoxin (LPS) has been associated indirectly with the reduction of ER stress. ATF6 null mice were highly susceptible to *Bacillus anthracis* and exerted increased bacterial load compared to the wild-type counteracts (Gade et al., 2012).

To investigate the effects of LPS (Sigma Aldrich, MO) in the IRE1α activation of the lungs, we measured the expression of phospho (p) IRE1α and IRE1α in wild-type mice treated for 24 h with either vehicle (saline) or LPS (intratracheally, 1.6 mg/kg). The Western Blot process was previously described (Uddin et al., 2020a). LPS (#L4130) was obtained from Sigma-Aldrich (St Louis, MO). The IRE-1α antibody (#3294 s) was purchased from Cell Signaling (Danvers, MA), and the phospho-IRE1α antibody (Ser724) (#PA1-16927) from Thermo Fisher Scientific (Waltham, MA). Seven weeks old animals were purchased from Envigo (Indianapolis, IN) and were maintained under pathogen-free conditions in a 12:12 h light:dark cycle. All procedures were evaluated and approved by the University of Louisiana Monroe IACUC. Our results (Fig. 1) indicate that LPS suppresses the activation of IRE1α, hence it suppresses at least one UPR branch (IRE1α) in the mice lungs.

Heat shock protein 90 (Hsp90) inhibitors are anti-cancer (Zuehlke et al., 2018; Neckers et al., 2018) and anti-inflammatory agents (Tukaj and Wegrzyń, 2016) which support the vascular barrier (Antonov et al., 2008; Chatterjee et al., 2007, 2008). They also induce the activation of UPR both *in vivo* and *in vitro* (Uddin et al., 2020b; Kubra et al., 2020b).

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**Disclosure of Competing Interest**

The author declare no conflicts of interest.

**References**

Akhter, M.S., et al., 2020a. Kifunensine compromises lung endothelial barrier function. Microvasc. Res. 132, 104051.

Akhter, M.S., et al., 2020b. Involvement of the unfolded protein response in the protective effects of growth hormone releasing hormone antagonists in the lungs. J. Cell Commun. Signal.

Antonov, A., et al., 2008. Heat shock protein 90 inhibitors protect and restore pulmonary endothelial barrier function. Am. J. Respir. Cell Mol. Biol. 39 (5), 551–559.

Aw, D., et al., 2020. Association of frailty with mortality in older inpatients with Covid-19: a cohort study. Age Ageing 49 (6), 915–922.

Bagrati, V., et al., 2019. Toll-like receptor 4 activation promotes multiple myeloma cell growth and survival via suppression of the endoplasmic reticulum stress factor Chop. Sci. Rep. 9 (1), 3245.

Barabutis, N., 2019. Unfolded protein response in acute respiratory distress syndrome. Lung 197 (6), 827–828.

Barabutis, N., 2020a. Heat shock protein 90 inhibition in the inflamed lungs. Cell Stress Chaperones 25 (2), 195–197.

Barabutis, N., 2020b. P53 in acute respiratory distress syndrome. Cell. Mol. Life Sci. 77 (22), 4725–4727.

Barabutis, N., Verin, A., Catravas, J.D., 2016. Regulation of pulmonary endothelial barrier function by kinases. Am. J. Physiol. Lung Cell Mol. Physiol. 311 (5), L532–L545.

Chan, Y.H., et al., 2019. Differential regulation of LPS-Mediated VE-Cadherin disruption in human endothelial cells and the underlying signaling pathways: a mini review. Front. Cell Dev. Biol. 7, 286.

Chatterjee, A., et al., 2007. Heat shock protein 90 inhibitors prolong survival, attenuate inflammation, and reduce lung injury in murine sepsis. Am. J. Respir. Crit. Care Med. 176 (7), 667–675.

Chatterjee, A., et al., 2008. Heat shock protein 90 inhibitors attenuate LPS-induced endothelial hyperpermeability. Am. J. Physiol. Lung Cell Mol. Physiol. 294 (4), 1755–63.

Gade, P., et al., 2012. An IFN-gamma-stimulated ATF6-C/EBP-beta-signaling pathway critical for the expression of Death Associated Protein Kinase 1 and induction of autophagy. Proc. Natl. Acad. Sci. U. S. A. 109 (26), 10316–10321.

Hetz, C., Axten, J.M., Patterson, J.B., 2019. Pharmacological targeting of the unfolded protein response for disease intervention. Nat. Chem. Biol. 15 (8), 764–775.

Hu, H., et al., 2018. The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection. Front. Immunol. 9, 3083.

Kubra, K.T., et al., 2020a. Luminisib competes with Kifunensine-induced lung endothelial barrier dysfunction. Curr. Drug Targets 21 (2), 218–229.

Kubra, K.T., et al., 2020b. Hsp90 inhibitors induce the unfolded protein response in bovine and mouse lung cells. Cell. Signal. 67, 109500.

Lu, Y.C., Yeh, W.C., Ohashi, P.S., 2008. LPS/TLR4 signal transduction pathway. Cytokine 42 (2), 145–151.

Neckers, L., et al., 2018. Methods to validate Hsp90 inhibitor specificity, to identify off-target effects, and to rethink approaches for further clinical development. Cell Stress Chaperones 23 (4), 467–482.

Nishitoh, H., 2012. CHOP is a multifunctional transcription factor in the ER stress response. J. Biochem. 151 (3), 217–219.

Soltanmohammadi, E., et al., 2021. Coordination in the unfolded protein response during aging in outbred deer mice. Exp. Gerontol. 144, 111191.

Takaji, S., Wegrzyn, G., 2016. Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. Cell Stress Chaperones 21 (2), 213–218.

Uddin, M.A., et al., 2020a. P53 deficiency potentiates LPS-Induced acute lung injury in vitro. Curr. Res. Physiol. 3, 30–33.

Uddin, M.A., et al., 2020b. Effects of heat shock protein 90 inhibition in the lungs. Med. Drug Discov. 6.

Woo, C.W., et al., 2009. Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by toll-like receptor signalling. Nat. Cell Biol. 11 (12), 1473–1480.

Zieberto, A.D., Moses, M.A., Neckers, L., 2018. Heat shock protein 90: its inhibition and function. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 373 (1738).

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