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Mechanisms of Ageing and Development 196 (2021) 111479

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Restoring the endothelial barrier function in the elderly

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ARTICLE INFO

Keywords:
Inflammation
Acute lung injury
Acute respiratory distress syndrome

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.

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 cofilin, 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 Wegrzyn, 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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https://doi.org/10.1016/j.mad.2021.111479
Received 25 January 2021; Received in revised form 24 February 2021; Accepted 26 March 2021
Available online 2 April 2021

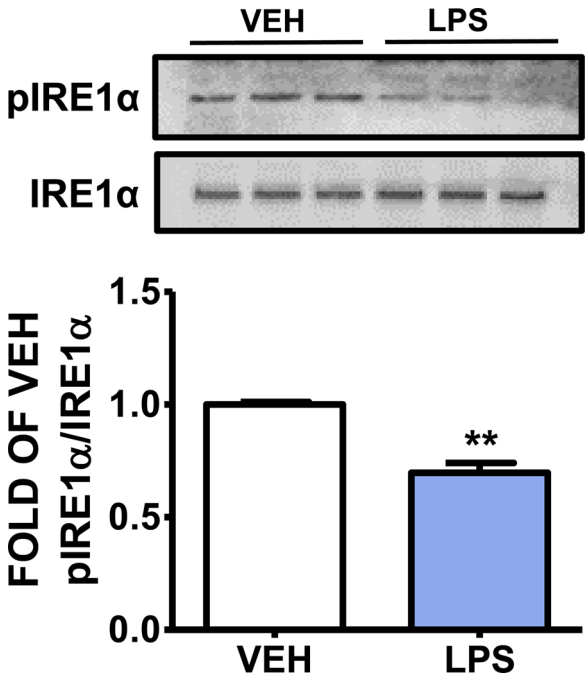

Fig. 1. LPS suppresses phospho-IRE1α in mice lungs.
Western Blot analysis of phosphorylated IRE1α (pIRE1α) and IRE1α in mice lungs treated with either vehicle (saline) or LPS (1.6 mg/kg) via an intra-tracheal injection for 24 h. The signal intensity of pIRE1α and IRE1α was analyzed by densitometry. Protein levels were normalized to IRE1α. Image J software (NIH) was used to perform densitometry of immunoblots. Graphpad Prism (version 5.01) was used to analyze the data. Student's *t*-test was performed to determine the statistical differences among groups. **P $<$ 0.01 vs vehicle (VEH), number of animals in each group = 3. Means ± SEM.

Hsp90 is a molecular chaperone which assists in the maturation of a plethora of inflammatory proteins (e.g. kinases, transcription factors) involved in human disease, including ARDS and sepsis (Barabutis, 2020a).

We examined whether Hsp90 inhibitors counteract the Kifunensine-induced endothelial hyper-permeability. AUY-922, a potent Hsp90 inhibitor, opposed the deteriorating effects of Kifunensine in the lung microvasculature, via UPR induction (Kubra et al., 2020a). UPR was also involved in the protective effects of Growth Hormone Releasing Hormone antagonists in vitro (Akhter et al., 2020b). Both compounds employ the endothelial defender and tumor suppressor P53 (Barabutis, 2020b). Laborious efforts are invested in the development of specific UPR activators and suppressors (Hetz et al., 2019). Hence, future studies employing endothelial specific mutations in mice will immerse into the unknown depths of the highly interrelated UPR universe, to investigate potential new therapies against endothelial barrier function-related pathologies.

Funding

Dr. Barabutis' research is supported by the R&D, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents through the Board of Regents Support Fund (LEQSF(2019-22)-RD-A-26) (PI: NB).

Declaration of Competing Interest

The author declare no conflicts of interest.

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