Hsp90 inhibition protects the hCMEC/D3 brain microvascular endothelial cells against oxidative stress

Mohammad Afaz Uddin, Mohammad Shohel Akhter, Khadeja-Tul Kubra, and Nektarios Barabutis

School of Basic Pharmaceutical and Toxicological Sciences, College of Pharmacy, University of Louisiana Monroe, Monroe, LA 71201, USA
Blood-brain barrier (BBB) dysfunction is associated with major neurological diseases, including cerebral ischemia/reperfusion (I/R) (stroke) and traumatic brain injury (TBI).

The development of new therapeutical approaches against these devastating disorders is urgent.

BBB endothelial cells interact with the surrounding cells of the CNS, including neurons and astrocytes. Those cells are important for maintaining brain homeostasis, and for preventing the entry of harmful substances.

Recent observations reveal that P53 regulates endothelial barrier function.

P53 supports endothelial barrier function by disrupting the inflammatory RhoA/MLC2 pathway and suppressing the actin-severing activity of cofilin.

Sweeney et al., *Nat Rev Neurol* (2018)
Hsp90 is a chaperone protein, involved in fundamental cellular processes including DNA repair, development, and immune responses.

Activation of Hsp90 has been linked to the development of multiple pathological conditions including cancer, inflammation, and viral infection.

Inhibition of Hsp90 is associated with anti-inflammatory responses.

P53 mediates the barrier enhancing effects of Hsp90 inhibitors, at least in part.

This transcription factor reduces APE1/Ref1 (ROS generator) in human tissues.

In our study we employed commercially available hCMEC/D3 cells from human temporal lobe microvessels to investigate the protective role of Hsp90 inhibition towards the H$_2$O$_2$-induced brain endothelium breakdown.

Uddin MA, Barabutis N., DNA Repair (Amst), 95:102952 (2020)
Methods

- Cell Culture
  - hCMEC/D3 cells

- In vitro treatments: H$_2$O$_2$, Hsp90 inhibitors (17-DMAG, AUY-922)

- Western Blot Analysis

- ROS measurement
  - H$_2$DCFDA

- Measurement of endothelial barrier function
  - ECIS-TEER measurements
Results

$\text{H}_2\text{O}_2$ induces brain endothelial barrier dysfunction

Hsp90 inhibition opposes the $\text{H}_2\text{O}_2$-induced P53 suppression
Results

Hsp90 inhibitors protect against H$_2$O$_2$-induced hCMEC/D3 barrier breakdown
Discussion and Conclusions

- Brain endothelial dysfunction contributes to the development and establishment of serious neurodegenerative diseases and cerebral disorders.

- Herein we report the capacity of Hsp90 inhibitors (P53 inducers) to support the brain endothelial function under severe oxidative conditions.

- Thus, we suggest that these anti-inflammatory agents may deliver new possibilities for the treatment of diseases related to brain endothelial dysfunction, including cerebral ischemia/reperfusion (I/R) (stroke), and traumatic brain injury (TBI).
Acknowledgement

This study was supported by 1) **NIGMS/NIH** (5 P20 GM103424-15 and 3 P20 GM103424-15S1), 2) 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) to N.B (PI), and 3) Malcolm Feist Partners Across Campuses Seed Program, Center for Cardiovascular Diseases and Sciences, LSUHS, Shreveport, LA 71103 (co-PI: N.B)

Bibliography