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### MEETING ABSTRACT

#### A1.4

##### **The role of biliverdin reductase in the protective activity of bilirubin in human endothelium**

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**Background:** Bilirubin is an endogenous antioxidant with anti-inflammatory and anti-thrombogenic activity. Recent studies showed that bilirubin is an essential antioxidant in the human endothelium. The current study aimed to determine the role of biliverdin reductase in the protective activity of bilirubin in the human endothelium.

**Methods:** Our experiments were done on the human endothelial cell line EA.hy926. Cells were exposed for 12 h to simulated oxidative stress conditions using the peroxy radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP). We studied the protective activity of bilirubin in the physiological concentration range of free unbound bilirubin (10–100 nM). To examine the role of BVR activity, we simultaneously incubated the endothelial cells with the BVR inhibitor apomorphine (10 nM). After 12 hours (at the end of oxidative stress), we determined the intracellular antioxidant activity of bilirubin using a CAA assay. We also measured the endothelial cell viability by resazurin-based fluorescent assay. To determine the cellular levels of bilirubin and biliverdin, we scraped and collected endothelial cells into methanol-DMSO solution, and analyzed them using HPLC-MS.

**Results:** Our experimental data confirmed that bilirubin acts as a strong intracellular antioxidant, and thus ameliorated the simulated oxidative-stress-induced injury. We discovered that inhibition by apomorphine of BVR decreased endothelial cell viability, decreased intracellular antioxidant activity, and lowered cellular levels of bilirubin.

**Discussion:** Our results suggest that BVR plays an essential role in bilirubin homeostasis in the human endothelium under increased oxidative stress. Endothelium exposed to high levels of ROS oxidizes more bilirubin to biliverdin, and then BVR plays an essential role in recycling biliverdin back to bilirubin. Our main finding is that inhibition of BVR diminished the protective activity of bilirubin on endothelial cells.

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**Keywords:** bilirubin – biliverdin – biliverdin reductase – endothelium – oxidative stress

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