**ABSTRACT**

**BACKGROUND AND PURPOSE:** Microvascular barrier breakdown is a hallmark of sepsis which associated with sepsis-induced multi-organ failure. Histidine-rich glycoprotein (HRG) is a 75-kDa plasma protein that was demonstrated to improve the survival of septic mice through regulation of cell shape, spontaneous ROS production in neutropils and adhesion of neutrophils to vascular endothelial cells. We investigated HRG's role in the LPS/TNF-α-induced barrier dysfunction of endothelial cells in vitro and in vivo and the possible mechanism, to clarify the definitive roles of HRG in sepsis.

**EXPERIMENTAL APPROACH:** EA.hy 926 endothelial cells were pretreated with HRG or human serum albumin before stimulation with LPS/TNF-α. A variety of biochemical assays were applied to explore the underlying molecular mechanisms how HRG protected the barrier function of vascular endothelium.

**KEY RESULTS:** Immunostaining results show HRG maintains the endothelial monolayer integrity by inhibiting cytoskeleton reorganization, losses of VE-cadherin and β-catenin, focal adhesion kinase degradation and cell detachment induced by LPS/TNF-α. HRG also inhibited the cytokine secretion from endothelial cells induced by LPS/TNF-α, which was associated with reduced NF-κB activation. Moreover, HRG effectively prevented the LPS/TNF-α-induced increase in capillary permeability in vitro and in vivo. Finally, western blot results demonstrated that HRG prevented the phosphorylation of mitogen-activated protein kinase family (MAPK) and RhoA activation, which are involved mainly in the regulation of cytoskeleton reorganization and barrier permeability.

**CONCLUSIONS:** Taken together, our results demonstrate that HRG has protective effects on vascular barrier function in vitro and in vivo which may be due to the inhibition of MAPK family and Rho activation.

**Key Words:** endothelial cells, barrier dysfunction, histidine-rich glycoprotein, sepsis