Honors Research Proposal

Regulation of Endothelial Function by Hydrogen Sulfide in Endotoxemia

11-11-14

Omar Glover
(800-567-624)

Supervised by Dr. Mark Clemens
Regulation of Endothelial Function by Hydrogen Sulfide in Endotoxemia

Specific Aims

Endotoxemia is a life threatening systemic inflammatory process caused by decreased tissue perfusion and oxygen delivery as a result of bacterial illnesses. Septic shock acts to disrupt hepatic microcirculatory thus leading to ischemia and subsequent hepatocellular damage. There is substantial evidence that this deregulation of hepatic microcirculation is a major factor in the onset of liver dysfunction in sepsis. This is characterized by an imbalanced production of vasoactive substances from parenchymal and nonparenchymal cells of the liver. Major vasoactive substances include endogenous gasotransmitters such as nitric oxide, carbon monoxide, and hydrogen sulfide which act as vasodilators or vasoconstrictors in hepatic tissue. Hydrogen sulfide is a known vasodilator that causes a protective effect within liver. However, our research suggests that in septic conditions it hydrogen sulfide exerts the opposite effect thus leading to a harmful proinflammatory response. As a result there is a change in microcirculation in hepatic endothelial cells that effect mitochondrial function. Reactive oxygen species, ROS is a major contributor to mitochondrial function. Studies show that H2S acts a reducing agent for ROS in mitochondria of endothelial cells. Furthermore, a proinflammatory response leads to increase neutrophils and cell adhesion interactions. Since hydrogen sulfide has been shown to affect mitochondrial function, we hypothesize that pretreatment with LPS will alter endothelial cell function with respect to mitochondrial membrane potential and reactive oxygen production. In addition, recent publications show that inhibition of hydrogen sulfide decreases neutrophil accumulation in vivo. Therefore, we hypothesize that this is the result of a change in the adherent property in endothelial cells. To evaluate these hypotheses, we propose the following aims:

Aim 1: To test whether endothelial cell function is altered in endotoxemia with respect to mitochondria membrane potential and reactive oxygen production.
   a) Evaluating mitochondrial membrane potential through fluorescence scope

Aim 2: To test whether the decrease in neutrophil accumulation by inhibition of hydrogen sulfide is due to an alteration in the adherent property of endothelial cells.
   a) Quantifying adherent cells

Background and Significance

The gaseous molecule of hydrogen sulfide is a well known toxic compound that pollutes the environment resulting in harmful health ailments affecting the cardiovascular, nervous, immune, and respiratory systems. However, it can also exert numerous physiological effects as a gaseous mediator, most notably as a vasodilator. The internal production of hydrogen sulfide
stems from L-cysteine by cystathionine β-synthase and/or cystathionine γ-lyase (CSE). Contrary to the well-known studies of hydrogen sulfide to be a vasodilator in cardio and nervous systems this study focuses on its protective effects of hydrogen sulfide on Human Microvascular Endothelial Cell (HMEC-1) function.

Previous studies indicate that hydrogen sulfide has anti-inflammatory effects in ischemic injury. This is due to its ability to prevent the adherence of leukocytes to respective interfaces on endothelium cells in addition to diminishing the assembly of inflammatory factors such as IL-1 β. Research shows that hydrogen sulfide inhibits the adhesion molecules ICAM-1 and V-CAM1, which are early markers for endothelial activation of the inflammatory response.

Yet, conflicting results have demonstrated hydrogen sulfide to produce a pro-inflammatory response under certain conditions. In a septic mouse, hydrogen sulfide acted as a pro-inflammatory factor as PAG, a hydrogen sulfide inhibitor, reduced leukocyte rolling and adherence with decreased mRNA and protein expression of ICAM-1, P-selectin, and E-selectin in the lungs and liver, while NaHS increased leukocyte rolling and attachment and up-regulated adhesion molecules. Likewise, in a mouse model of acute pancreatitis, PAG suppressed the production of substance-p, expression of preprotachykinin-A and neurokinin-1 receptor in acinar cells. However, this is not the case when knockout mouse are subjected to endotoxemia. It elicits a pro-inflammatory response caused by the deregulation of the microcirculatory system in liver endothelial tissue. As a result, different results suggest that hydrogen sulfide role in inflammation may be cell-specific and disease-specific, but the exact mechanism through which hydrogen sulfide exercises needs further research.

This upcoming year I will further investigate the hydrogen sulfide story. By understanding the mechanisms involved in the inflammation response during sepsis conditions we can determine a therapeutic way to prolong liver function during sepsis. Not only is hydrogen sulfide found in hepatic endothelial cells, but also in the nervous system, heart, lungs, proximal gastrointestinal tract, limbs, teeth, skin and even tumor cells. The liver plays a crucial role in filtering toxins from the blood. Maintaining its function is critical to the recovery of patients and will allow other parts of the body the necessary time to repair. Lastly, this research will be the first of its kind to look specifically at the mechanisms behind the hydrogen sulfide pathway under septic conditions.

**Approach**

**Aim 1:** To test whether endothelial cell function is altered in endotoxemia with respect to mitochondria membrane potential and reactive oxygen production.

**Overall Rational:** Pretreatment with LPS will alter endothelial cell function with respect to mitochondrial membrane potential and reactive oxygen production. We expect that without LPS pretreatment, ET-1 will stimulate reactive oxygen production by mitochondria in HMEC-1 and depolarization of the mitochondria and H2S treatment will ameliorate this. We expect that
LPS pretreatment will result in loss of protection by H2S or even potentiation of the inflammatory effect.

a) HMECs will be treated with LPS or vehicle for 6 or 24 hours. Cells will then be loaded with rhodamine123 (fluorescent dye that measures mitochondrial membrane potential) and propidium iodide (marker for cell death) for 10 minutes loading. Baseline will be recorded on fluorescent scope for 10 minutes followed by addition of Na2S for 10 minutes ET-1 for 15 minutes. Mitochondrial membrane potential and number of dead cells will be quantified.

**Statistical Analysis**

We will use a two way ANOVA with response to endothelin being the measured variable and LPS and H2S as the two factors.

**Aim 2:** To test whether the decrease in neutrophil accumulation by inhibition of hydrogen sulfide is due to an alteration in the adherent property of endothelial cells.

**Overall Rational:** Hydrogen sulfide decreases neutrophils because of a change in the adherent property in endothelial cells. We expect that treatment with H2S alone will decrease adhesion, but treatment with H2S after LPS would increase adhesion.

a) HMECs will be treated with LPS for 6 or 24 hours with or without GYY4137 (slow releasing hydrogen sulfide donor). Fluorescently labeled THP-1 cells (human monocyte cell line) will be added followed by incubation for 15 minutes. Non-adherent cells will be removed and the remaining adherent cells will be counted on a fluorescent scope. We will then treat HMECs with LPS for 6 or 24 hours with or without propargylglycine (cystathionine gamma lyase inhibitor, which prevents hydrogen sulfide production). Fluorescently labeled THP-1 cells (human monocyte cell line) will be added followed by incubation for 15 minutes. Non-adherent cells will be removed and the remaining adherent cells will be counted on a fluorescent scope.

**Statistical Analysis**

We will use a two way ANOVA with response to number of adherent cells being the measured variable and LPS and H2S as the two factors.

**Sources:**

1. Ciro Coletta, Andreas Papapetropoulos, Katalin Erdelyi, Gabor Olah, Katalin Modis, Panagiotis Panopoulos, Antonia Asimakopoulou, Domokos Gero, Iraida Sharina, Emil Martin, Csaba Szabo, Hydorgen sulfide and nitric oxide are mutally dependent in the
regulation of angiogenesis and endothelium-dependent vasorelaxation, *PNAS*. 2012, **109**, (23), 9161-9166


