Hydrogen sulfide, another simple gas with complex biology

Christopher Guettler and Paul Kubes

Calvin, Phoebe & Joan Snyder Institute for Chronic Diseases, University of Calgary, Calgary, Alberta, Canada; Department of Critical Care Medicine, University of Calgary, Calgary, Alberta, Canada; and Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta, Canada

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BACTERIAL INFECTIONS have been one of the major scourges of humankind throughout history. Sepsis is recognized as a global health problem with an increasing incidence (5). The rate of hospitalization for sepsis more than doubled between 2000 and 2008 in the US (16). No monotherapy has been successful in improving mortality, including the recent failure of recombinant activated protein C to show efficacy in clinical trials (21). Although early antibiotic intervention is critical, the rise of antibiotic-resistant bacteria underscores the importance of understanding this syndrome. In addition to infection, the immune system is thought to be hyperactivated early on and the cardiovascular system appears to be altered, causing ischemia and underperfusion. The impairment of these systems may lead to multiple organ failure that is the hallmark feature of sepsis.

In this issue of American Journal of Physiology Gastrointestinal and Liver Physiology Norris et al. (36) set out to examine the potential dysregulation of blood flow to the liver and the role of hydrogen sulfide (H$_2$S) in a rodent endotoxemia model. Interestingly, sepsis-associated liver injury is a predictive sign of poor prognosis (23). Since the liver is crucial for metabolic and immunological homeostasis (39), any functional impairment to the liver is likely to have wide ramifications for the course of disease.

Although the original discovery that the host produces a gas, nitric oxide (NO), that could generate toxic derivatives, was met with some skepticism, the identification of multiple enzyme systems that mediate this event has paved the road of acceptance for the discovery of other gaseous substances including carbon monoxide (CO) and most recently H$_2$S, both of which are known environmental pollutants. However, it is now recognized that H$_2$S is a blood flow regulator in addition to being an endogenous modulator of inflammation, as well as a modulator of neuronal activity (22), blood pressure regulator (55), and an inducer of insulin secretion (56). Many of these effects are reported to involve H$_2$S directly increasing K$_{ATP}$ channel currents (56, 61). The main enzymes responsible for its biosynthesis are cystathionine-γ-lyase (CSE) and cystathionine-β-synthetase, although others have been implicated (28, 42). Clearly, much like the complexity of nitric oxide biology, H$_2$S also has multiple biological actions.

The role of H$_2$S in the regulation of hepatic perfusion was first investigated by Fiorucci et al. (12). They were able to demonstrate that CSE is expressed in hepatocytes and hepatic stellate cells (HSCs) also known as Ito cells, but importantly not in the sinusoidal endothelial cells (SECs). Portal infusion of an H$_2$S donor, NaHS, antagonized increased hepatic resistance induced by norepinephrine infusion in a K$_{ATP}$ channel-dependent manner. From this work, it seemed reasonable to conclude that endogenous H$_2$S would function as a vasodilator, preserving blood flow. This raised the question why the inhibition of endogenous H$_2$S via propargylglycine (PAG) decreased hepatic dysfunction in endotoxemia (59). Previous work by Norris et al. (37) indicated that H$_2$S might have differential effects on presinusoidal and sinusoidal sites within the liver.

In this issue, Norris et al. (36) expand on their previous work and study the effect of endogenous H$_2$S on the hepatic microcirculation under basal conditions and in a model of endotoxemia using very sophisticated intravital microscopy. They demonstrate that portal infusion of the H$_2$S donor Na$_2$S elicits a small but significant decrease in sinusoidal diameter and an increase in diameter variability. During endotoxemia it is well known that the liver microvasculature becomes very sensitive to vasoconstrictors including endothelin. Inhibition of endogenous H$_2$S in endotoxemic rats via PAG decreased the LPS-induced microcirculatory hypersensitivity to endothelin-1 (ET-1), leading to an attenuated ET-1-induced increase in portal pressure and an abrogation of sinusoidal constriction. This had significant physiological consequences since blockade with PAG improved tissue oxygen delivery measured by a very stylish technique: NADH autofluorescence.

The regulation of hepatic microvascular blood flow is very complex because the liver consists of both a venous and arterial source of blood leading to the liver. Multiple vasoregulators and an intricate interplay of HSCs, Kupffer cells (KCs), and SECs contribute to hepatic blood flow regulation. In the classic paradigm ET-1, NO, and CO are kept in a balanced state to maintain a normal sinusoidal perfusion under basal conditions. Endothelial ET-1 binds to the ET$_A$ receptor, which is primarily expressed by HSCs (17). These cells surround the sinusoids and are thought to contract, thus reducing blood flow. Binding to ET$_B$$_1$ receptors expressed by SECs leads to the activation of endothelial nitric oxide synthase (eNOS) and ensuing relaxation. Basal CO levels are maintained by the constitutively expressed parenchymal heme oxygenase-2 (HO-2). NO and CO both interact with the soluble guanylyl cyclase, leading to an increase in intracellular cGMP. NO has been shown to limit the release of ET-1 and to increase the expression of endothelial HO-1 (41). This balance is disturbed in the state of inflammation. Six hours after LPS injection there is a marked increase in the hepatic mRNA level of inducible nitric oxide synthase (iNOS), ET-1, the ET$_A$ receptor, the ET$_B$ receptor, and HO-1 (45). A shift from vasodilating ET$_A$ to vasoconstricting ET$_B$$_2$ receptors has been proposed (1). Additionally, endotoxin suppresses ET-1-mediated eNOS activation through Cav-1. It is thought that low-level NO produced by eNOS protects the liver microcirculation in sepsis, whereas the strong...
upregulation of iNOS contributes to sinusoidal dysfunction (51). Conversely, CO inhibits macrophage iNOS expression (51) and ET-1 (34). Clearly additional work will be needed to fully elucidate the complicated interactions that occur between the three gases CO, NO, and H2S in blood flow regulation of the liver microcirculation.

The liver exhibits a further complexity of blood flow regulation between the arterial and venous inflow. The total inflow of blood by the hepatic artery and portal vein is controlled via the hepatic arterial buffer response. It takes place in the periportal space of Mall. A decrease in portal flow is met with a vasodilatation of the hepatic artery (25). Adenosine is thought to be the main but not sole mediator of this vasodilation (31) in a process referred to as the “washout hypothesis.” There is also evidence for a role of NO, CO, and even H2S, as well as the neurotransmitters calcitonin gene-related peptide and neuropeptide in modulating the hepatic arterial buffer response (4, 11, 38, 44).

Damage to the hepatic parenchyma can occur as a result of several intertwined mechanisms. Sinusoidal underperfusion results in a mismatch between oxygen or nutrient supply and demand, leading to hepatic injury (26, 48). In a rat ischemia-reperfusion model microcirculatory dysfunction directly correlated with increased serum aspartate aminotransferase and alanine aminotransferase levels and reduction of bile flow (47). Additionally, direct cytotoxicity can be elicited via increased levels of systemic cytokines like TNF-α (50), causing hepatocyte apoptosis or aperiocrosis (43). Finally, the recruitment of primarily neutrophils into the endotoxemic liver can also cause hepatic toxicity. Neutrophils have been shown to adhere avidly in the inflamed liver in a CD44/hyaluronan-dependent manner and this occluded about 30% of sinusoids leading to some hepatoxicity (32). Blocking CD44 detached the neutrophils and unplugged the sinusoids. Neutrophils have been shown to also adhere directly to hepatocytes via ICAM-1/Mac-1 (15, 20, 27) and release their arsenal of antimicrobial/cytotoxic mediators including reactive oxygen intermediates and proteases, causing hepatic injury. Recently it has been shown that platelets bind neutrophils and induce them to release all of their nuclear chromatin and granular contents also known as neutrophil extracellular traps (NETs) (33). Xu et al. (54) demonstrated that when histones, one of the major cytopoetic components of NETs, were neutralized, this reduced endothelial injury and the mortality of mice in LPS, TNF, and cecal ligation and puncture (CLP) models of sepsis.

Elevated levels of H2S have been observed in various inflammatory states, and it is principally considered proinflammatory although some exceptions have been noted (53). The reversibility CSE inhibitor PAG has been shown to improve the outcome in models of pancreatitis (3), burn injury (60), and sepsis (59), whereas in a model of trinitrobenzene sulfonic acid-induced colitis it increased inflammation and mortality (49).

An unexpected finding in this study was the direct antiadhesive effect of H2S on neutrophils. The authors postulate that the mechanism of neutrophil accumulation in the sinusoids in endotoxemia in part depends on a sinusoidal narrowing (19) and thus the improved perfusion with PAG may release neutrophils from the sinusoids. However, other studies have reported that even a large number of adherent neutrophils in sinusoids do not necessarily block perfusion (13), which calls this dependence of sinusoidal narrowing on neutrophil adhesive effect into question. It has been reported that PAG treatment of CLP-induced sepsis reduces mRNA and protein levels of ICAM-1, P-selectin, E-selectin, IL-1, IL-6, TNF-α, CCL2, and CXCL2 in lung and liver (57, 58). In vitro treatment with PAG decreases the primarily neutrophil chemokine receptor CXCR2 by enhancing CXCL2-induced CXCR2 internalization and desensitization (8). Patrick et al. (40) demonstrated in an adipovirus-mediated gene transfer model of TNF-α that blocking either CXCL1 or CXCL2 reduces the number of neutrophil adhering in sinusoids. Treatment with H2S increases CXCR2 expression on neutrophils, thus facilitating their CXCL2-directed chemotaxis (58). H2S may have far-reaching effects on the inflammatory response, and these changes may also contribute to the decreased neutrophil sequestration after PAG administration. Clearly, additional studies are needed to elucidate the underlying antiadhesive mechanisms of H2S inhibition.

The hepatic response to most insults (such as ischemia-reperfusion injury, endotoxemia, and acetaminophen-induced hepatic intoxication) also involves the activation of resident cells including KCs, HSCs, and SECs. KCs are the largest population of resident tissue macrophages in the body (35). In addition to their critical role in catching bacteria, upon TLR4 stimulation by LPS, KCs respond by releasing TNF-α, IL-1β, IL-8, IL-12, IL-18, G-CSF, and GM-CSF and a wide range of chemokines (9, 10). Interestingly, Fukuda et al. (14) showed that the gadolinium chloride-mediated inactivation of KCs resulted in a marked decrease in neutrophil recruitment, oxidant production, and less damage to the SECs, the cellular component known to be the most susceptible to injury by reactive oxygen species (7). These studies demonstrate that KCs play a role in the initial step of neutrophil recruitment and subsequent damage to the hepatic parenchyma in response to LPS and raise the possibility that H2S does not necessarily directly affect neutrophil recruitment but could dampen KC responses in endotoxemic liver (18). Intriguingly, the endothelium expresses functional TLR4/CD14 (46) and can thus react to LPS as well. Therefore the effect of H2S on this cell type should also be investigated.

H2S research is still in its infancy, limited by the availability of optimal reagents. Current limitations include PAG nephrotoxicity (24) and lack of specificity (6). The development of slow-releasing H2S donors like GYY4137 (30) might shed new light on the physiological role of H2S. Studies using slow-releasing donors intriguingly show an anti-inflammatory effect (2, 29, 52). Nevertheless, this study provides compelling new evidence for why PAG is hepatoprotective in endotoxemia by improving perfusion and inhibiting the potentially detrimental neutrophil infiltration. The unraveling of the “third gas” H2S has just begun and may share with the other gaseous moieties both complexity of biological actions but also importance in biological processes in the liver microcirculation.

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