Journal of Pediatric Surgery 53 (2018) 1111–1117

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

# The route and timing of hydrogen sulfide therapy critically impacts intestinal recovery following ischemia and reperfusion injury☆

Amanda R. Jensen [a,c], Natalie A. Drucker [a,c], Jan P. te Winkel [a,c], Michael J. Ferkowicz [a,c], Troy A. Markel [a,b,c,*]

[a] Department of Surgery, Section of Pediatric Surgery, Indianapolis, IN
[b] Riley Hospital for Children at Indiana University Health, Indianapolis, IN
[c] The Indiana University School of Medicine, Indianapolis, IN

ARTICLE INFO

*Article history:*
Received 20 February 2018
Accepted 27 February 2018

*Key words:*
Hydrogen sulfide
GYY4137
Intestinal ischemia
Perfusion
Inflammation

ABSTRACT

*Purpose:* Hydrogen sulfide ($H_2S$) has many beneficial properties and may serve as a novel treatment in patients suffering from intestinal ischemia–reperfusion injury (I/R). The purpose of this study was to examine the method of delivery and timing of administration of $H_2S$ for intestinal therapy during ischemic injury. We hypothesized that 1) route of administration of hydrogen sulfide would impact intestinal recovery following acute mesenteric ischemia and 2) preischemic $H_2S$ conditioning using the optimal mode of administration as determined above would provide superior protection compared to postischemic application.

*Methods:* Male C57BL/6J mice underwent intestinal ischemia by temporary occlusion of the superior mesenteric artery. Following ischemia, animals were treated according to one of the following (N = 6 per group): intraperitoneal or intravenous injection of GYY4137 ($H_2S$-releasing donor, 50 mg/kg in PBS), vehicle, inhalation of oxygen only, inhalation of 80 ppm hydrogen sulfide gas. Following 24-h recovery, perfusion was assessed via laser Doppler imaging, and animals were euthanized. Perfusion and histology data were assessed, and terminal ileum samples were analyzed for cytokine production following ischemia. Once the optimal route of administration was determined, preischemic conditioning with $H_2S$ was undertaken using that route of administration. All data were analyzed using Mann–Whitney. P-values $<0.05$ were significant.

*Results:* Mesenteric perfusion following intestinal I/R was superior in mice treated with intraperitoneal (IP) GYY4137 (IP vehicle: $25.6 \pm 6.0$ vs. IP GYY4137: $79.7 \pm 15.1$; $p = 0.02$) or intravenous (IV) GYY4137 (IV vehicle: $36.3 \pm 5.9$ vs. IV GYY4137: $100.7 \pm 34.0$; $p = 0.03$). This benefit was not observed with inhaled $H_2S$ gas (O2 vehicle: $66.6 \pm 11.4$ vs. $H_2S$ gas: $81.8 \pm 6.0$; $p = 0.31$). However, histological architecture was only preserved with intraperitoneal administration of GYY4127 (IP vehicle: $3.4 \pm 0.4$ vs. IP GYY4137: $2 \pm 0.3$; $p = 0.02$). Additionally, IP GYY4137 allowed for significant attenuation of inflammatory chemokine production of IL-6, IP-10 and MIP-2. We then analyzed whether there was a difference between pre- and postischemic administration of IP GYY4137. We found that preconditioning of animals with intraperitoneal GYY4137 only added minor improvements in outcomes compared to postischemic application.

*Conclusion:* Therapeutic benefits of $H_2S$ are superior with intraperitoneal application of an $H_2S$ donor compared to other administration routes. Additionally, while intraperitoneal treatment in both the pre- and postischemic period is beneficial, preischemic application of an $H_2S$ donor was found to be slightly better. Further studies are needed to examine long term outcomes and further mechanisms of action prior to widespread clinical application.

*Type of study:* Basic science.
*Level of evidence:* N/A



Acute mesenteric ischemia (AMI) is a devastating disease that occurs when the blood supply to the intestine is cut off abruptly. The lack of blood flow to the small intestine leads to ischemia, cellular damage, intestinal necrosis and death if left untreated. Despite advances in medical care, mortality rates remain as high as 55%–80% [1,2]. In the pediatric population, intestinal ischemia can readily be observed with malrotation and midgut volvulus, incarcerated hernias, or with adhesive bowel obstructions [3]. Intestinal ischemia can also be seen in

☆ This publication was made possible with support from:1) KL2TR001106 (A. Shekhar, PI) from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award2) Indiana University Health, Indianapolis, IN.

* Corresponding author at: Indiana University School of Medicine, Riley Hospital for Children at IU Health, 705 Riley Hospital Drive, Suite RI 2500, Indianapolis, IN 46202. Tel.: +1 317 437 2506; fax: +1 317 274 4491.
*E-mail address:* tmarkel@iupui.edu (T.A. Markel).

https://doi.org/10.1016/j.jpedsurg.2018.02.072
0022-3468/

other disease pathologies such as congenital heart disease, fibromuscular dysplasia, abdominal compartment syndrome, or aortic thrombosis, to name a few [4]. Currently there are no medical therapies that allow for salvage of the ischemic intestine. Patients that require small bowel resection can often require long term total parenteral nutrition or intestinal transplantation secondary to short gut syndrome. AMI causes significant morbidity and mortality; therefore, new treatment modalities are urgently needed. The discovery and development of new medical therapies to improve intestinal perfusion and decrease cellular compromise would drastically change the medical management of this devastating disease.

One drug that could potentially provide protection in the setting of intestinal ischemia is hydrogen sulfide. Hydrogen sulfide ($H_2S$) is an endogenously produced gasotransmitter that plays an integral role in many physiological and pathological processes. It is known to regulate cell death and apoptosis, reduce inflammatory processes and provide cytoprotection [5,6]. It also is known to play a key role in vascular relaxation and angiogenesis [7–9]. Studies in the literature have found it to be protective in the setting of cerebral, cardiac, hepatic, renal and intestinal ischemia–reperfusion injuries [10,11].

Hydrogen sulfide also plays key roles in the production and modulation of several cytokines and chemokines. Two such chemokines include macrophage inflammatory protein 2(MIP-2) and interferon gamma-induced protein 10 (IP-10). These two chemokines play a prominent role in neutrophil recruitment in the inflammatory response following intestinal ischemia [12–14]. Additionally, interleukin 6 (IL-6), an acute phase reactant, has been found to promote intestinal hyperplasia and villous growth, while interleukin 10 (IL-10), a key anti-inflammatory cytokine, has been found to be dramatically decreased in the setting of intestinal ischemia [12,14–16]. $H_2S$ has also been found to induce angiogenesis and vasodilation through vascular endothelial growth factor (VEGF) mediated mechanisms [7].

Prior to widespread therapeutic use, the optimal mode of delivery must be identified. Differences in administration between inhaled hydrogen sulfide gas, direct intraperitoneal application, and intravenous therapy could affect the benefits observed with $H_2S$ treatment. Previous studies have observed benefits with all routes of administration but a study to determine the most efficacious route of administration has not previously been undertaken [17–19]. Furthermore, it is unclear if hydrogen sulfide preconditioning can further improve outcomes. If there is benefit to prophylactic administration of $H_2S$ prior to any ischemic episode, $H_2S$ could prove to be a novel preventative therapy for those patients at risk for intestinal ischemia. The purpose of this small scale pilot study was to examine mode of delivery and timing of administration during intestinal ischemic injury. We hypothesized that: 1) route of administration of hydrogen sulfide would impact intestinal recovery following acute mesenteric ischemia and 2) preischemic $H_2S$ conditioning using the optimal mode of administration as determined above would provide superior protection compared to postischemic application.

## 1. Methods

### 1.1. Animals

The Indiana University Institutional Animal Care and Use Committee approved all experimental protocols and animal use. Male adult wild-type C57BL/6J mice used in this study underwent at least 48 h of acclimation prior to any experimentation. Normal chow and water were provided and all mice were kept in 12-h light/dark cycled housing. For all animal experiments there were six mice per group.

### 1.2. Ischemia–reperfusion model

Mice were anesthetized using 3% isoflurane followed by maintenance at 1.5% isoflurane in oxygen. Temperature homeostasis was achieved through use of a heating pad and the abdomen was prepped through hair removal and sterile preparation with 70% ethanol followed by betadine. One milliliter of 0.9% normal saline was injected subcutaneously in all mice preoperatively to account for intraoperative fluid losses. Postoperative pain was managed with preoperative subcutaneous administration of analgesia (1 mg/kg buprenorphine and 5 mg/kg carprofen).

Under sterile conditions, a midline laparotomy was performed and the intestines were eviscerated. The base of the superior mesenteric artery was identified and clamped using an atraumatic microvascular clamp. The intestines were then placed back into the abdominal cavity and the abdomen was temporarily closed using silk suture to prevent evaporative losses. Following 60 min of intestinal ischemia, the abdomen was reopened and the atraumatic clamp was removed. The abdominal fascia and skin were then closed in a two-layer fashion with silk suture. Following surgery, animals were placed in warm cage and allowed to recover. Once fully awake and alert, animals were returned to animal housing.

### 1.3. $H_2S$ administration

In order to determine optimum mode of administration, $H_2S$ was administered by the following routes: inhaled, intravenous, or intraperitoneal. A total of six mice were used in each treatment group. Treatments were administered in the postischemic period as previously established by our lab [20]. Treatment groups ($N = 6$) included: 1) $O_2$ only (systemic control, 4 L/min), 2) $H_2S$ Gas, (80 ppm at 2 L/min mixed with 2 L/min oxygen), 3) intravenous (IV) PBS (40 μL; IV vehicle control), 4) IV GYY4137 (a slow-releasing $H_2S$ donor; 50 mg/kg in 40 μL of PBS), 5) intraperitoneal PBS (IP; 250 μL; IP vehicle control), or 6) IP GYY4137 (a slow-releasing $H_2S$ donor; 50 mg/kg in 250 μL of PBS). The $O_2$ vehicle and $H_2S$ gas were administered for one hour following removal of the atraumatic clamp on the SMA. In the intravenous and intraperitoneal therapy groups, treatment was administered immediately after clamp removal.

Once the optimum route of therapy was identified (intraperitoneal, see Results below) we performed preconditioning experiments of hydrogen sulfide using this route, and compared preischemic application to postischemic application ($N = 6$/group): 1) IP PBS (250 μL; IP vehicle control), and 2) IP GYY4137 (a slow-releasing $H_2S$ donor; 50 mg/kg in 250 μL of PBS). Animals in the preischemia treatment groups were given vehicle or $H_2S$ therapy one hour prior to ischemia.

### 1.4. Perfusion analysis

Intestinal mesenteric perfusion was analyzed using a Laser Doppler perfusion Imager (LDI; Moor Instruments, Wilmington, DE). Perfusion images were acquired at baseline, at initial clamping of the superior mesenteric artery and at 24 h following intestinal ischemia. Using images obtained, a region of interest was created around the entirety of exposed intestines. Using three images from each time point, a flux mean perfusion was acquired for the region of interest. Perfusion was expressed as a percentage of baseline (mean ± SEM). After the 24-h recovery analysis, mice were euthanized with isoflurane overdose and cervical dislocation. Intestinal tissues were explanted for further analyses.

### 1.5. Intestinal histological injury evaluation

At sacrifice, terminal ileum specimens were harvested, fixed in 4% paraformaldehyde, embedded in paraffin and sectioned for 2 μm thickness. Slides were subsequently stained with hematoxylin and eosin. A histological scoring method of intestinal damage was used as previously described: 0, no damage; 1, subepithelial space at the villous tip; 2, loss of mucosal lining at the villous tip; 3, loss of less than half of the villous structure; 4, loss of more than half of the villous structure; and 5, transmural necrosis [21]. All histological sections were evaluated by two blinded authors (NAD, JPW) and scores were averaged.