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Protection Against Vaso-occlusion with Daily Dosing of HBI-002, a Low Dose Oral Carbon Monoxide Formulation, in Sickle Cell Disease Mouse Models

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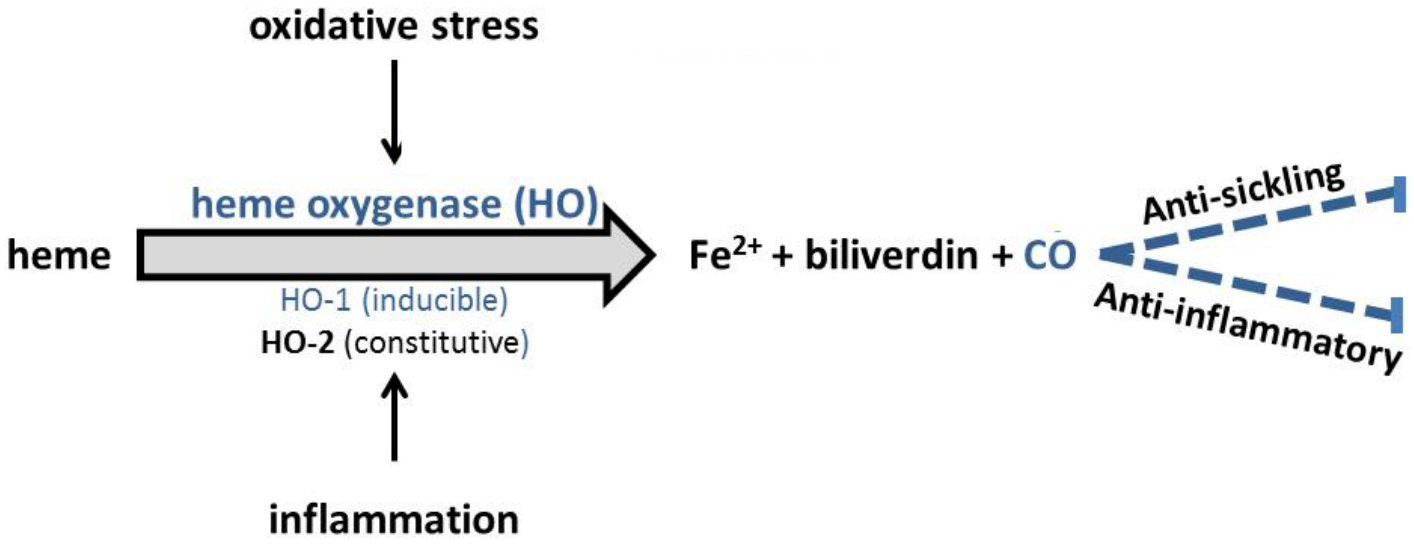
Heme Oxygenase (HO): A Critical Protective Pathway

HO is a stress response gene that provides critical cytoprotection.

- Generates carbon monoxide (CO)
- CO mimics the cytoprotection of heme oxygenase
- Highly novel dual mode of action in SCD: anti-sickling and anti-inflammatory



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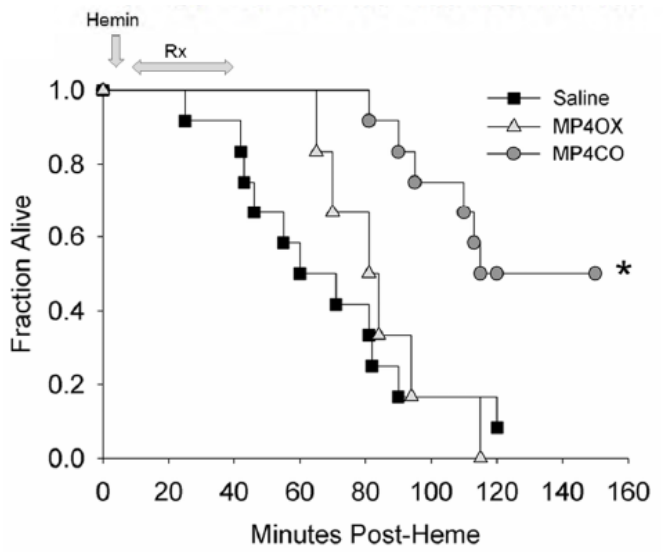
See: Gomperts E, Belcher JD, Otterbein LE, Coates TD, et al. The Role of Carbon Monoxide and Heme Oxygenase in the Prevention of Sickle Cell Disease Vaso-Occlusive Crises. Am J Hematol. 2017.

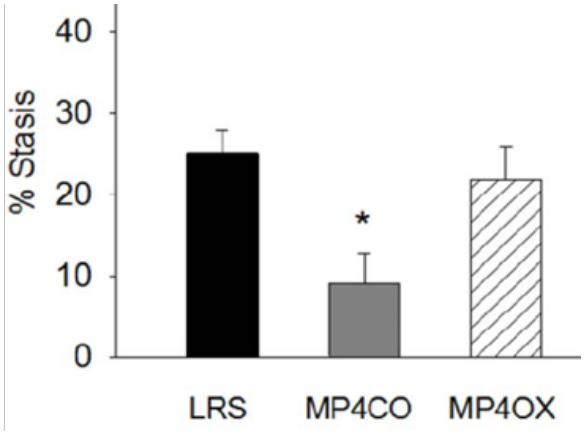
Key **Preclinical** Findings of Low Dose CO Research in SCD

- In vitro
  - Low dose CO prevents sickle cell formation in red blood cells from a SCD patient (Sirs, 1963)
  - Low dose CO was shown to melt HbS polymers (Aroutiounian, 2001)
- Preclinical in vivo
  - Prolonged exposure to low dose inhaled CO significantly reduced leukocytosis and liver pathology and inflammation in transgenic SCD mice (Beckman, 2009)
  - Low dose inhaled CO prevented vascular stasis and leukocyte adhesion in transgenic SCD mice (Belcher, 2006)
  - Administration of low dose CO (PEG-Hb-based CORM MP4CO) inhibited the effects of stimuli that induce vaso-occlusive crises and improved mortality in transgenic SCD mice (Belcher, 2013)

Survival after hemin infusion (i.v.)



Stasis after hypoxic stimulus



Source: Belcher et al 2013

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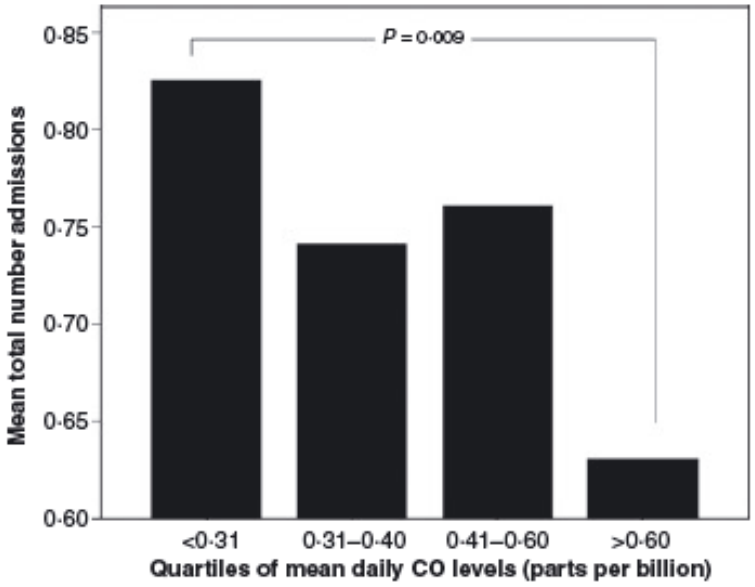
Key **Clinical** Findings of Low Dose CO Research in SCD

- Administration of inhaled CO to a SCD patient reduced the proportion of sickled red blood cells (Sirs, 1963)
- Beutler, et al found that administration of inhaled CO to two SCD patients prolonged red blood cell survival (Beutler, 1975)
- Yallop et. al. found that higher atmospheric levels of CO correlated with a lower hospital admission rate for sickle cell crisis (Yallop, 2007)

Clinical evidence for CO-mediated prevention of sickle cell crises in SCD patients



Source: Yallop et al, 2007

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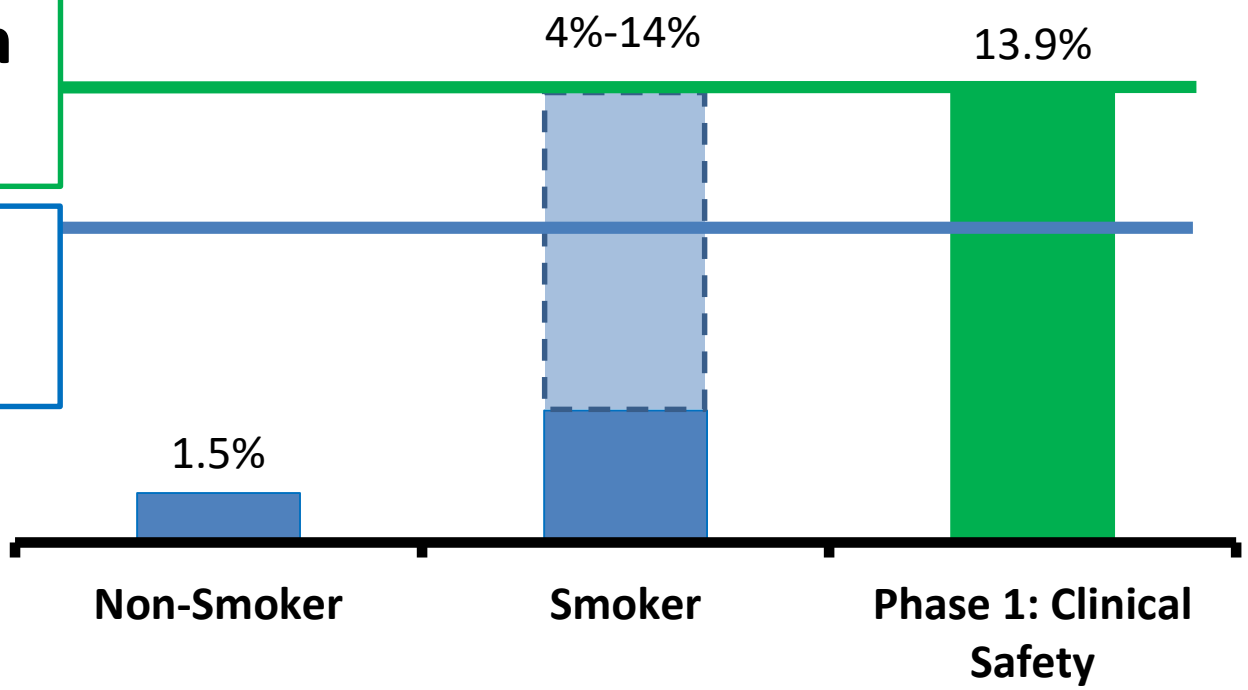
CO Is Produced Physiologically: Key Is To Stay at Safe, Therapeutic Levels How Low is Safe?

Minor adverse effects at COHb% of ~20%

Clinical safety shown at ~14% COHb

HBI-002 targets less than 10% COHb



COHb levels of 14% or less appear to have no deleterious effect

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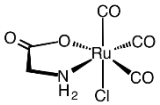
Sources: CDC, WHO

CO delivery modalities

CO Product	Advantages	Barriers to Use
 CO gas	• Quick uptake in lung	• Accidental inhalation -CO canisters • Dosing inaccuracy
 CO carrier	• Potential oral pill	• Carrier molecule toxicity • CO release kinetics, stability
 CO in water	• Oral liquid • Precise CO absorption	• Low solubility of CO • Stomach volume precludes use
 HBI-002	• Oral liquid • Prompt CO absorption • **Dosed by volume targeting both efficacy and safety**	• **No barriers**

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HBI-002: Oral Carbon Monoxide (CO) Therapeutic

The Problem: **haven't been able to use low dose CO in the prevention of VOC's in SCD because of delivery**

HBI-002, an oral liquid therapeutic, **solves the delivery problem, enabling chronic use as a therapeutic in SCD**

Lead Indication:

- Sickle Cell Disease: Chronic use for SCD crisis **prevention**

Description

- A liquid solution containing CO
- Designed for oral CO delivery
- Contains generally recognized as safe (GRAS) excipients
- Filled in packaging appropriate for oral consumption
- Secured intellectual property

HBI-002: Additional Safety Measures

- HBI-002 is administered as a **single oral dose per 24 hours**: Peak CO achieved with rapid fall-off as absorbed CO is eliminated via **expiration**
- HBI-002 dose volume designed to **limit the potential for overdosing** based on maximum stomach capacity
- COHb blood gas level measurement is a standard clin lab procedure with rapid turnaround. Also measured using pulse oximetry

Sickle Cell Disease: HBI-002 Mechanisms of Action Address the Key Underlying Aspects of the Disease

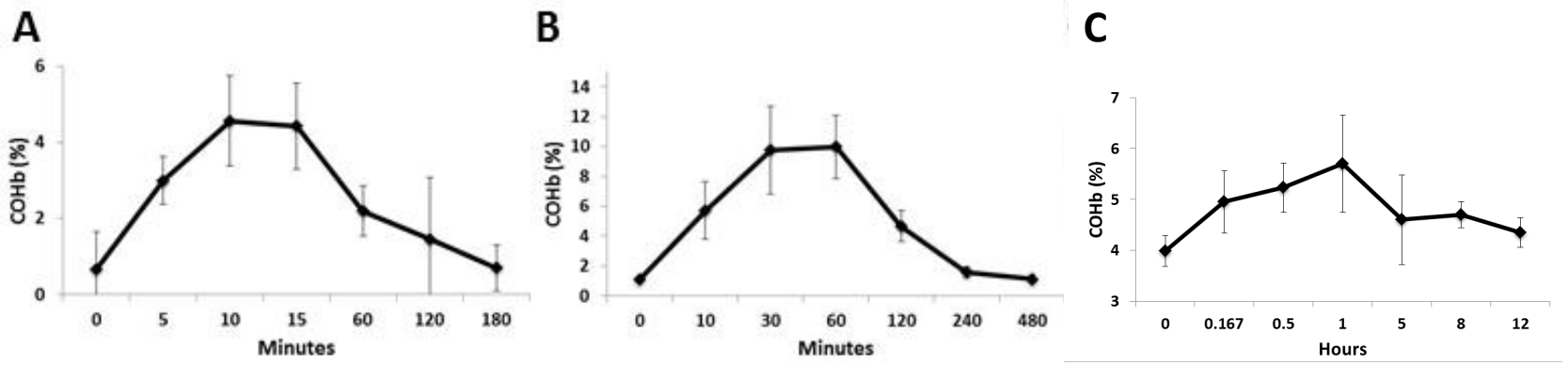
- Mechanism 1: Prevention of polymerization
  - CO binds tightly to hemoglobin locking it into a conformation that is unable to polymerize into long rigid polymers (tactoids)
  - This prevents red blood cells from sickling

- Mechanism 2: Anti-inflammatory
  - CO down-regulates key genes associated with inflammation (e.g. NF-κB)
  - CO up-regulates key genes associated with limiting inflammation (e.g. HO-1, Nrf2)
  - Inflammation is limited by preventing the obstruction and ischemia from sickled red cells
  - Anti-apoptotic

Demonstrated Bioavailable CO from Oral HBI-002 in Normal Mice, Rats, and Dogs

Pharmacokinetic studies in normal mice (**A**), normal rats (**B**), and dogs (**C**) demonstrate:

- Rapid uptake of CO into blood occurs from oral administration of HBI-002
- The ability to reach peak potentially therapeutic levels of CO-Hb
- No adverse signs associated with the administered HBI-002 were observed in any animal



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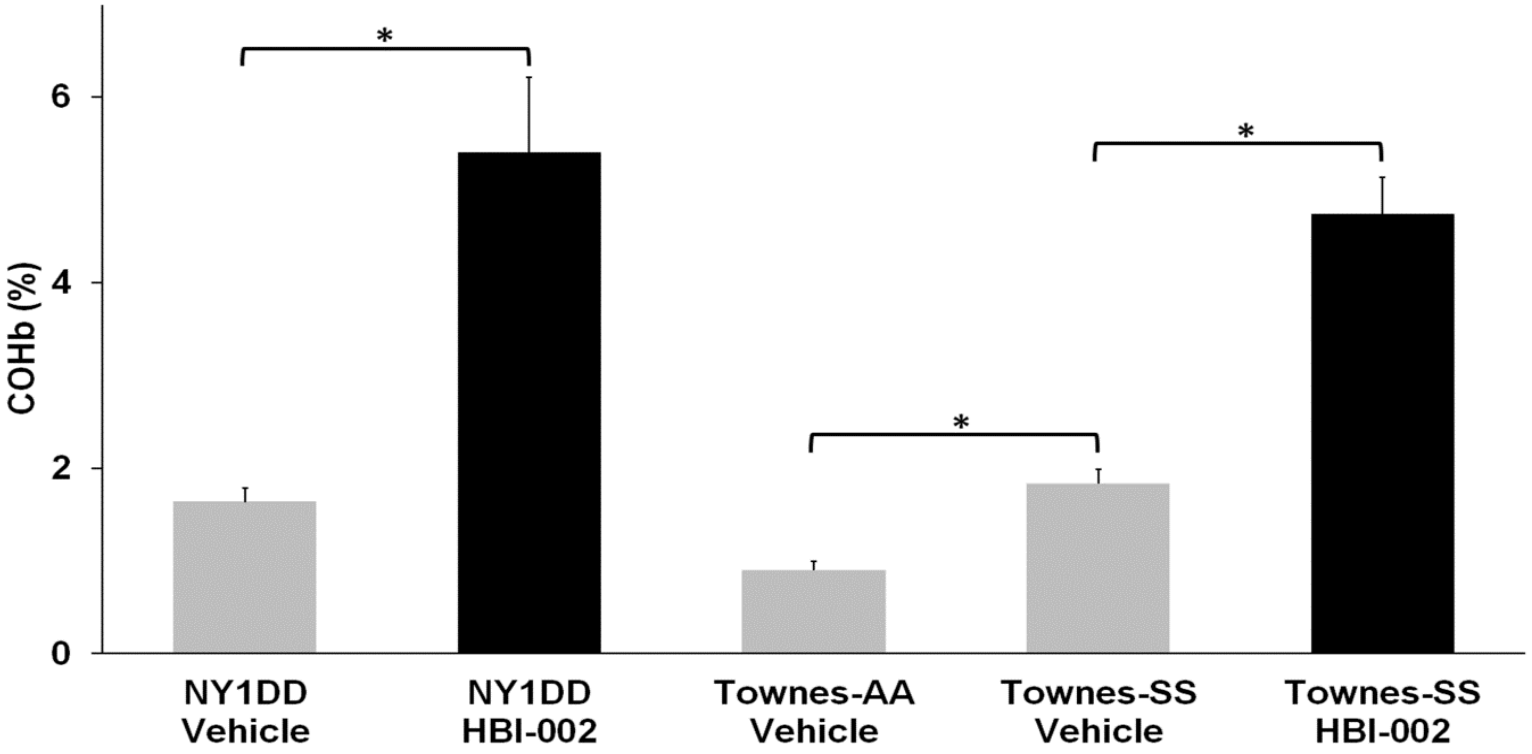
Preclinical Efficacy in SCD Mice Study Design

- Seven groups of male and female SCD mice (n=6 per group; HBI-002:placebo 1:1)
  - 5 groups NY1DD mice (all VOC model)
  - 2 groups of TOWNES mice (one VOC model, one inflammation/hemolysis outcomes)
- Dosing regimens (oral gavage HBI-002 or placebo (HBI-002 vehicle))
  - Dosing once per day
  - NY1DD mice (VOC model dosing prior to hypoxia): 1 hr; 1 day; 3 days; 5 days; 10 days
  - TOWNES mice: Same regimen as tested in NY1DD mouse VOC model but five days of dosing
  - Second group of Townes mice dosed for 10 days for inflammation/hemolysis outcomes
- VOC Model Procedure
  - Mice implanted with dorsal skin-fold chambers.
  - Immediately before hypoxia, 20-25 flowing venules were selected and mapped.
  - Mice were subjected to one hour of hypoxia (7% O2), followed by re-oxygenation in room air.
  - All venules were re-examined for blood flow at 1, 2, 3 and 4 hours after hypoxia. The number of static (no flow) venules were counted and % stasis was calculated.
- Inflammation/Hemolysis Outcomes Procedure
  - Daily dosing for 10 days, assessments on Day 10 (Townes mice only)
- Readouts
  - Bioavailable CO: COHb
  - Vaso-occlusion: % stasis
  - Inflammation: WBC counts, NF-κB, VCAM-1, HO-1, and Nrf2
  - Hemolysis: RBC, hematocrit, Hb, reticulocytes

COHb Level After a Single Oral Gavage of HBI-002 (at 5 Minutes Post-Dose)



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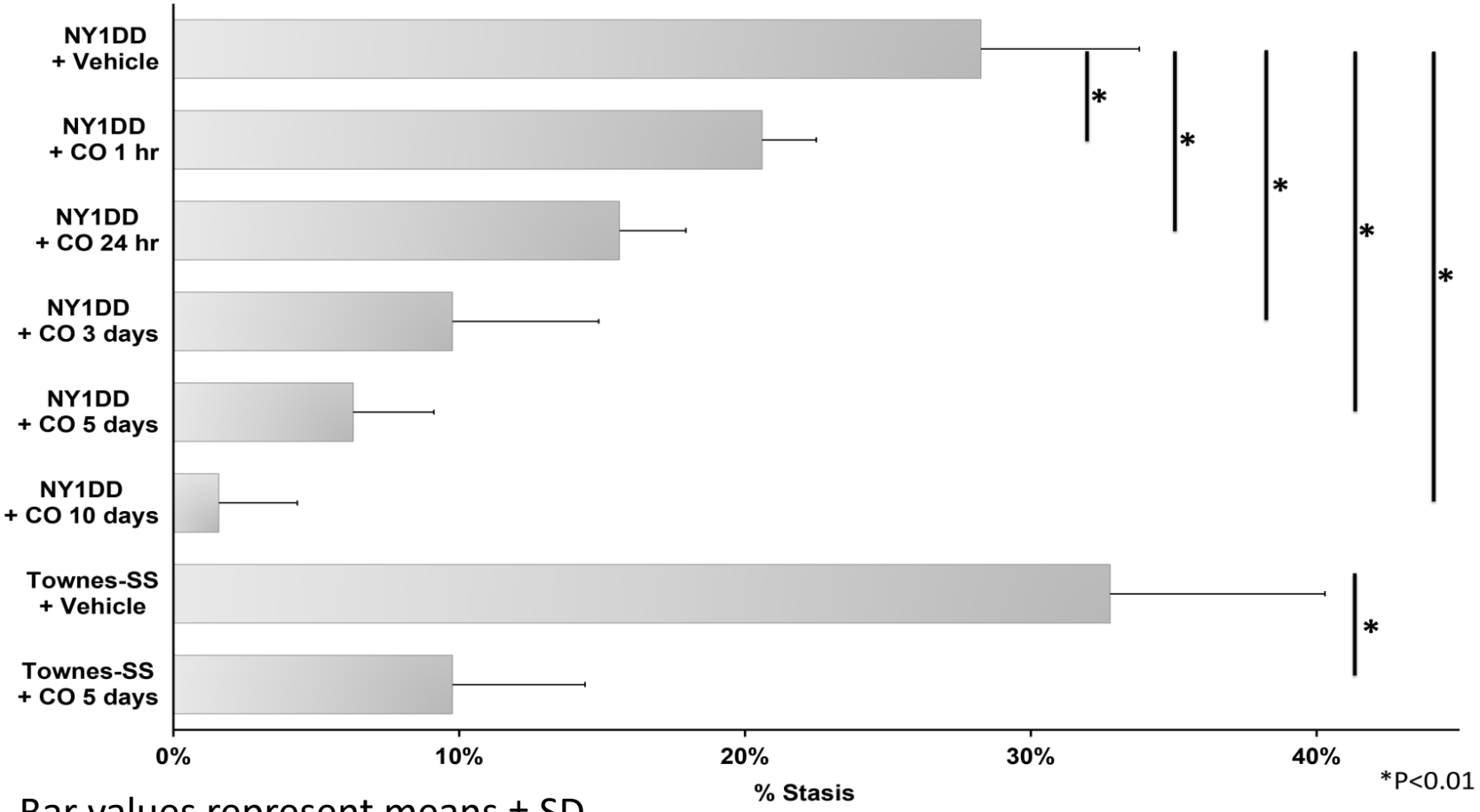
HBI-002 Provides Protection Against Vascular Stasis; Degree of Protection Increases with Days of Dosing



Bar values represent means ± SD.

*P<0.01, HBI-002 vs. vehicle.

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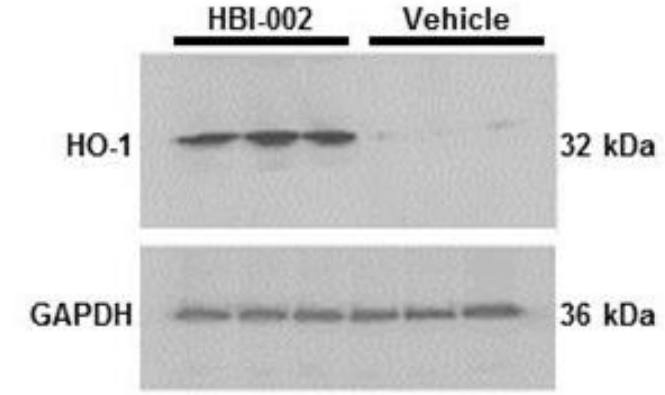
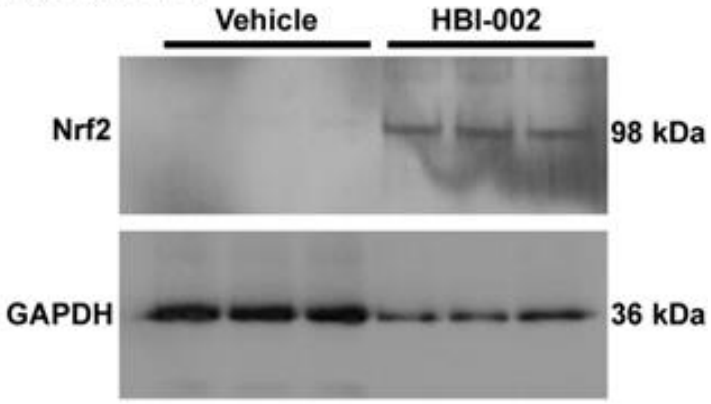
HBI-002 Improves Markers of Cytoprotection and Reduces Markers of Inflammation in TOWNES SCD Mice After 10 Days of Dosing

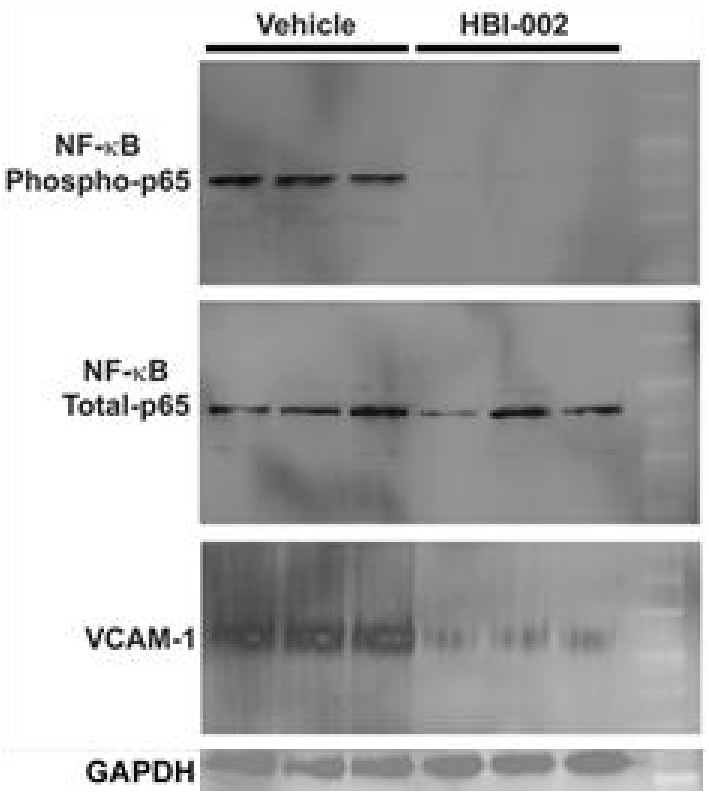
HO-1



Nrf2



NF-κB and VCAM-1



- Nrf2 and HO-1 expression was examined on immunoblots of hepatic nuclear extracts and microsomes, respectively
- NF-κB phospho-p65 and VCAM-1 expression was examined on immunoblots of hepatic nuclear extracts and microsomes, respectively

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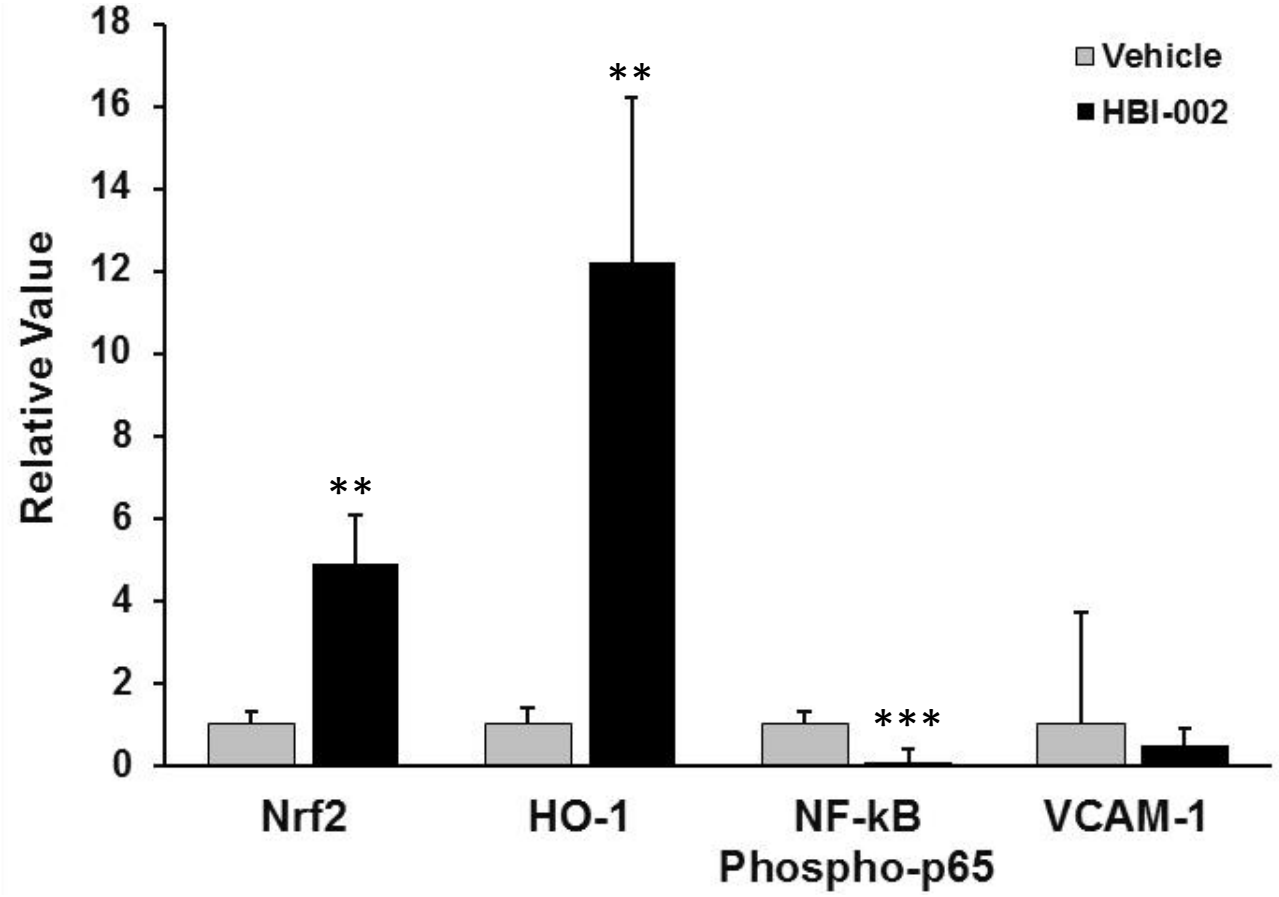
Quantification of Markers of Cytoprotection and Inflammation in Townes SCD Mice After 10 Days of Dosing



Relative values to vehicle value set at 1.0; Bar values represent means ± SD.
**P<0.01, ***P<0.001 HBI-002 vs. vehicle.

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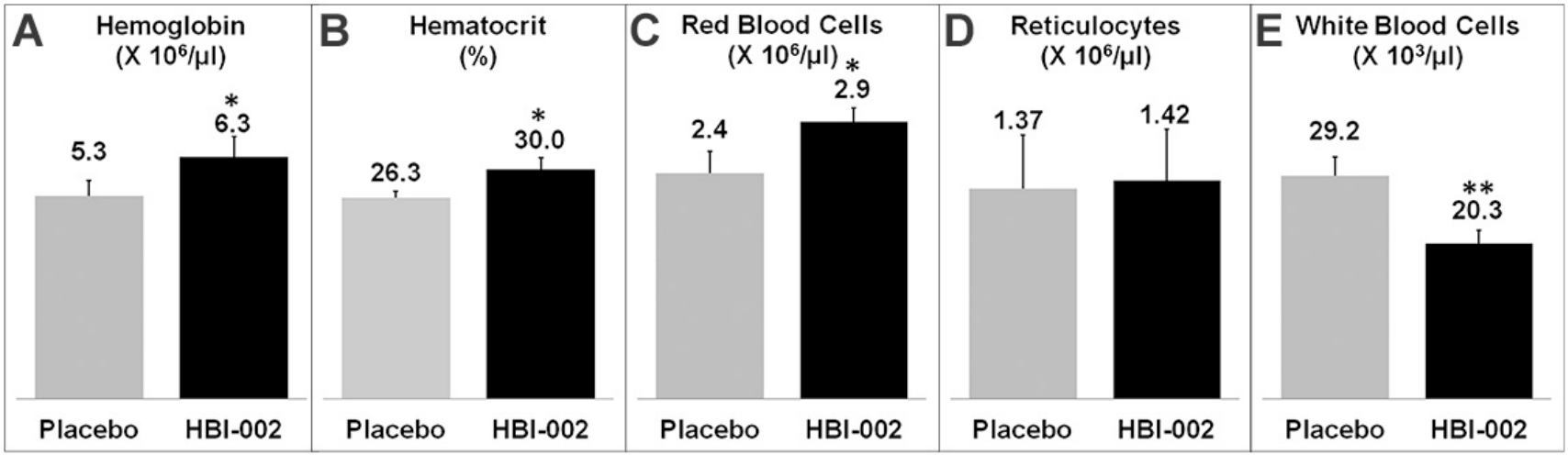
HBI-002 Improves Markers of Hemolysis/Sickling (A-D) and Inflammation (E) in TOWNES SCD mice After 10 Days of Dosing



Bar values represent means ± SD.
*P<0.05 and **P<0.01, HBI-002 vs. vehicle.

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HBI-002 Development Status

- Appropriate pharmacokinetics demonstrated
- Completed IND-enabling preclinical toxicology studies
- cGMP manufacturing in place
- IND and Phase 1 study in healthy volunteers planned

Conclusions

- HBI-002 **improves red cell parameters** without an increase in reticulocytes and **decreases the WBC count** with **daily oral dosing**
- HBI-002 **improves the inflammatory response** in sickle cell mice by enhancing HO-1, Nrf2 and limiting NFκB expression in liver tissue with daily oral dosing
- Single oral dose HBI-002 administration **inhibits vaso-occlusion** to a similar degree in SCD mice as inhaled CO and CO-PEGHb (MP4CO) in published reports
- **The degree of inhibition of vaso-occlusion increases progressively to low numbers with increasing daily oral dosing**