Effects of Oral Carbon Monoxide on Doxorubicin-Induced Cardiotoxicity

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#P391

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Financial Disclosure

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President and CEO of Hillhurst Biopharmaceuticals Inc.

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BACKGROUND/OBJECTIVE

- **Doxorubicin** (DOX) carry adverse effects such as cardiotoxicity[1,2];

- Iron plays a key role in DOX cardiotoxicity and free **Heme** is toxic and a major source of redox-active iron[3,4];

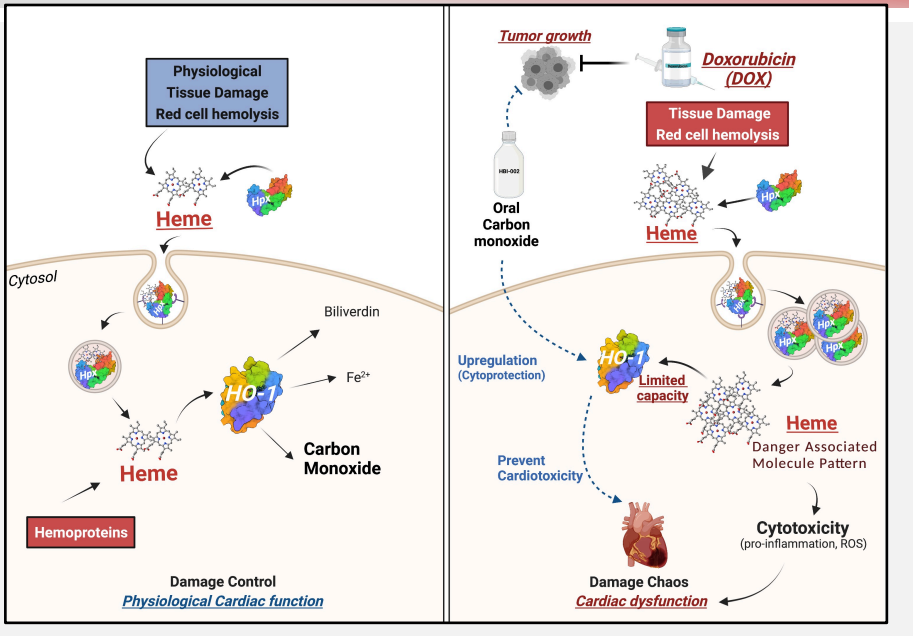
- To limit free Heme toxicity, Heme is metabolized by **Heme Oxygenase-1 (HO-1)**, a known cardioprotective enzyme, into iron, bilirubin, and **carbon monoxide (CO)**.

- CO presents a promising approach to preventing anthracycline cardiotoxicity[5-7]. Inhaled CO or carrier molecule-bound CO, present substantial barriers to use;

- HBI-002 (Hillhurst Biopharmaceuticals, CA): oral CO drug produces COHb well below levels known to be associated with toxicity and amenable to administration of CO as a drink[8];

- Hypothesis: a sudden elevation in Heme contributes significantly to DOX-induced cardiotoxicity;

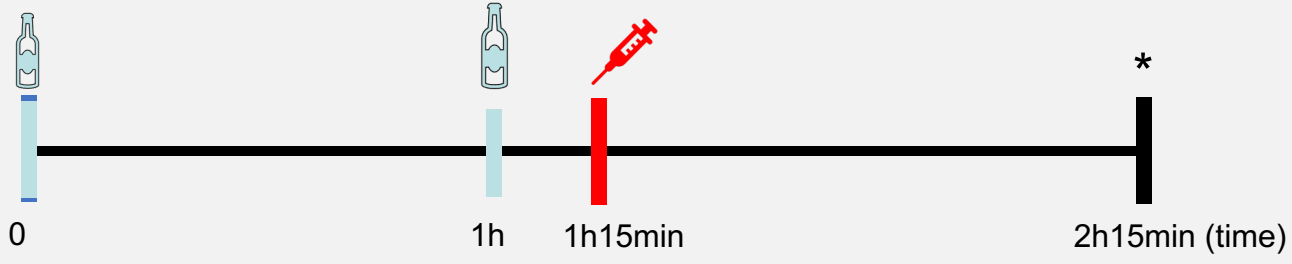
- Objective: test if HBI-002 attenuates DOX-induced cardiotoxicity



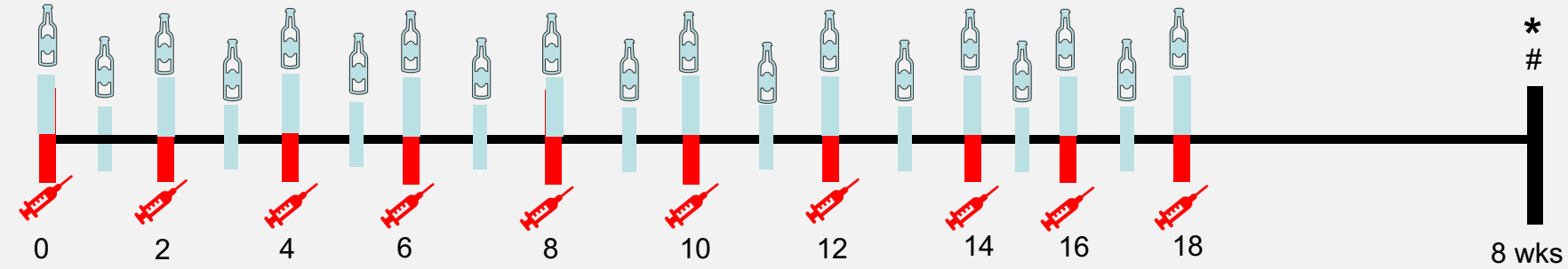
Proposed model for how free heme could participate in DOX-induced cardiotoxicity and how the oral CO administration could modulate heme-oxygenase-1 (HO-1) to protect the heart and prevent tumor growth. Tissue injury leads to the sudden release of cellular contents, including heme, toxic to the cells. To avoid heme toxicity, it is rapidly scavenged by Hemopexin (Hpx) and endocytosed. Heme is then metabolized by Heme Oxygenase-1 (HO-1), a known cardioprotective gene that catabolizes heme into three bioactive products: iron, biliverdin, and carbon monoxide (CO). HO-1 expression and the subsequent generation of endogenous CO and/or exogenous CO can induce beneficial effects (left panel). In the right panel, DOX treatment results in elevated circulating heme levels, which reach the heart inducing damage in myocardial cells. Moreover, DOX-induced heme release could promote carcinogenesis. Oral CO may be used as a strategy of cardioprotection against DOX toxicity by upregulation of HO-1 and to prevent cancer progression. Created using BioRender (https://biorender.com).

METHODS AND MATERIALS

1 – Dox acute protocol



2 – Dox chronic protocol



C57BL/6: male, 10-12 wks old

HBI-002 (10ml/kg, p.o.)

Vehicle (10ml/kg, p.o.)

Two doses ~1h apart

Doxorubicin

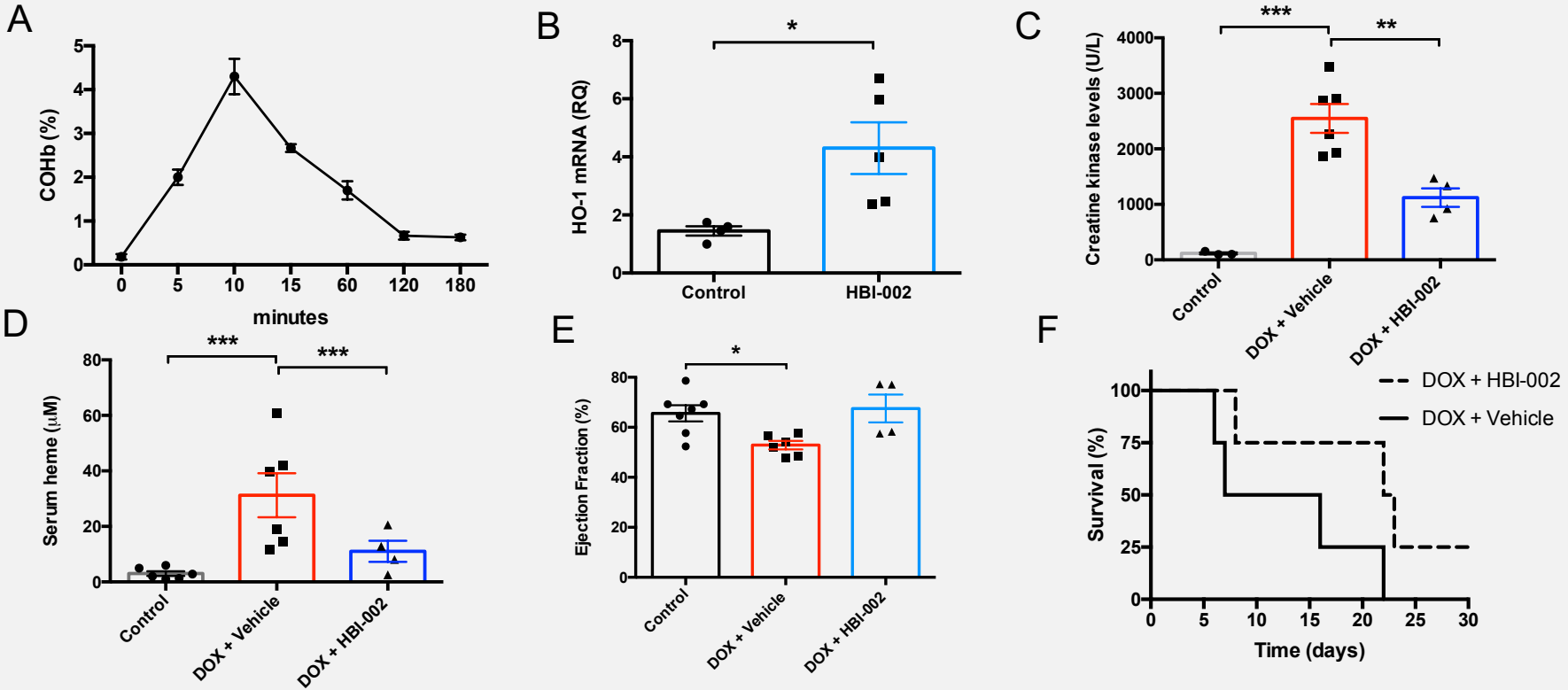
Acute: 1 time: 20mg/kg, i.p.

Chronic: 10 times: 2mg/kg, i.p.)

\# Echocardiography

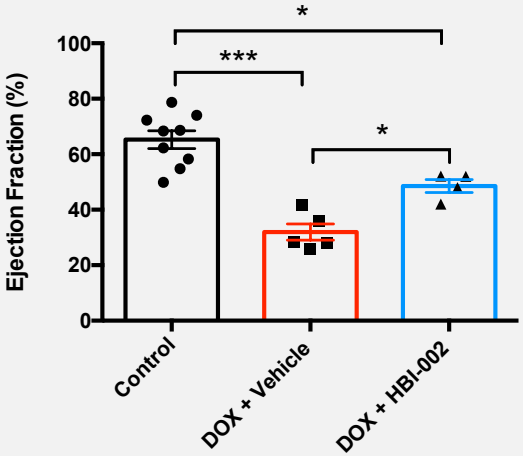
* Sacrifice – blood and tissue harvest

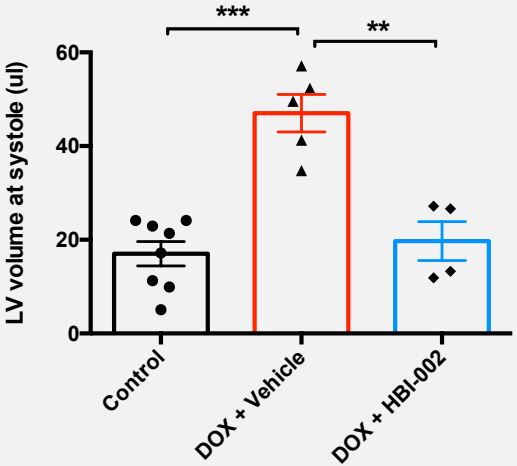
HBI-002 - DOX ACUTE EFFECTS

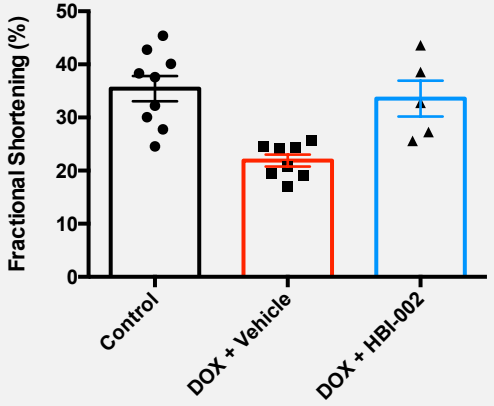


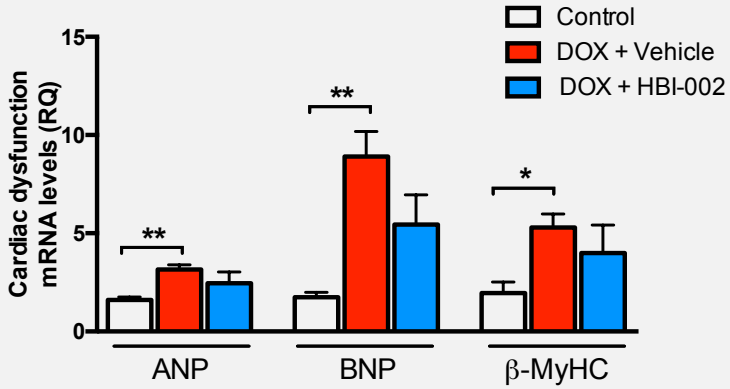
HBI-002 prevented short and mid-term effects of single dose of DOX. **A**. HBI-002 increases COHb levels in mice. **B.** Ten days of HBI-002 upregulates HO-1 mRNA levels in the heart. HBI-002 attenuated the increase in serum creatine kinase (**C**) and heme levels (**D**), after one hour of a single dose of DOX (20 mg/kg, i.p.). **E.** HBI-002 prevented DOX-induced cardiac injury (**E**) and mortality (**F)** in mice. Mean ± SEM; * p<0.05, **p<0.01, ***p<0.001. n=3-7 mice/group.

HBI-002 – DOX CHRONIC CARDIOTOXIC EFFECTS



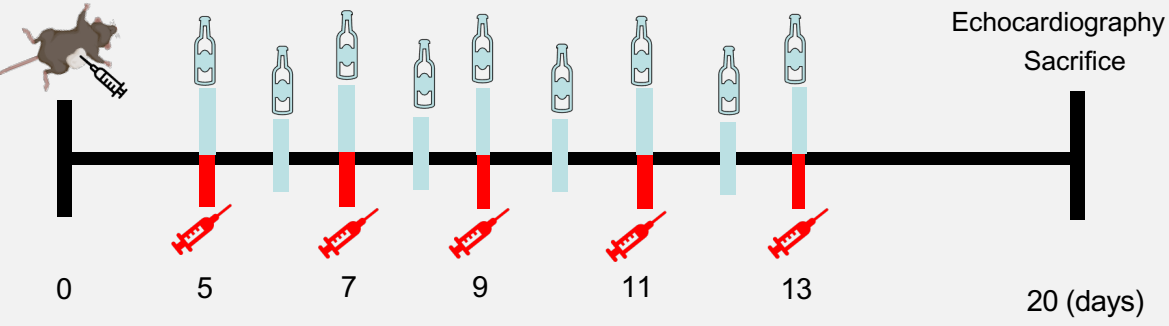






HBI-002 attenuated long-term (8 weeks) cardiotoxic effects of DOX. HBI-002 chronic dose regimen prevented long-term DOX-induced cardiac injury. Cardiac dysfunction markers – ANP (atrial natriuretic peptide), BNP (brain natriuretic peptide), β-MyHC (cardiac beta-myosin heavy chain). Mean ± SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$. n=3-7 mice/group.

HBI-002 – Tumor Growth



C57BL/6 male, 10-12 wks old

Lewis lung carcinoma (LL/2) - 10^6 cells

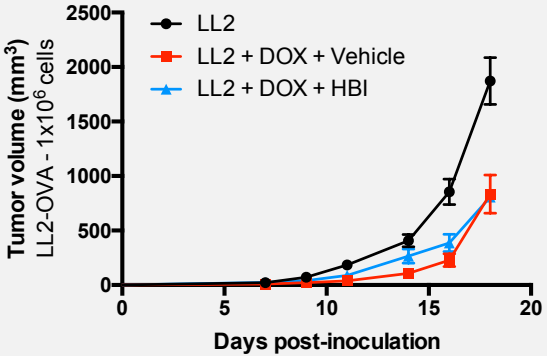
HBI-002 - 10ml/kg, p.o.

Vehicle - 10ml/kg, p.o.

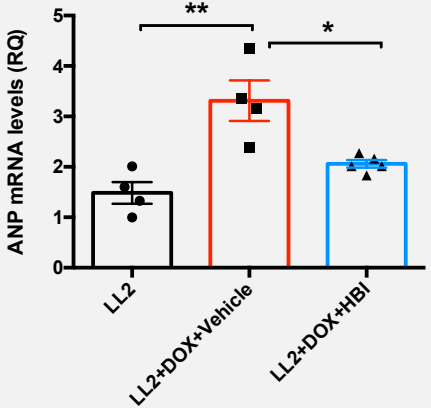
Two doses

Doxorubicin (4mg/kg, i.p.)

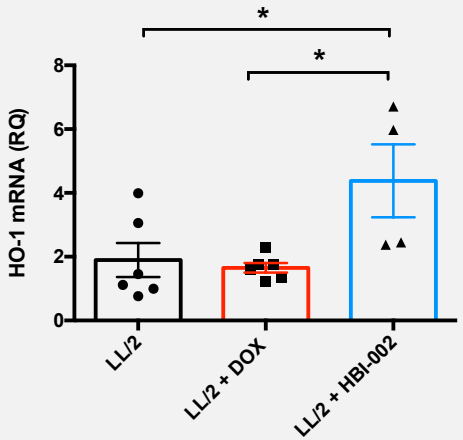
Tumor growth



Cardiac dysfunction marker



HO-1 upregulation



HBI-002 does not affect DOX chemotherapy and promotes cardioprotection possibly by HO-1 overexpression in the heart. Mean ± SEM; * p<0.05, **p<0.01. n=3-6 mice/group.

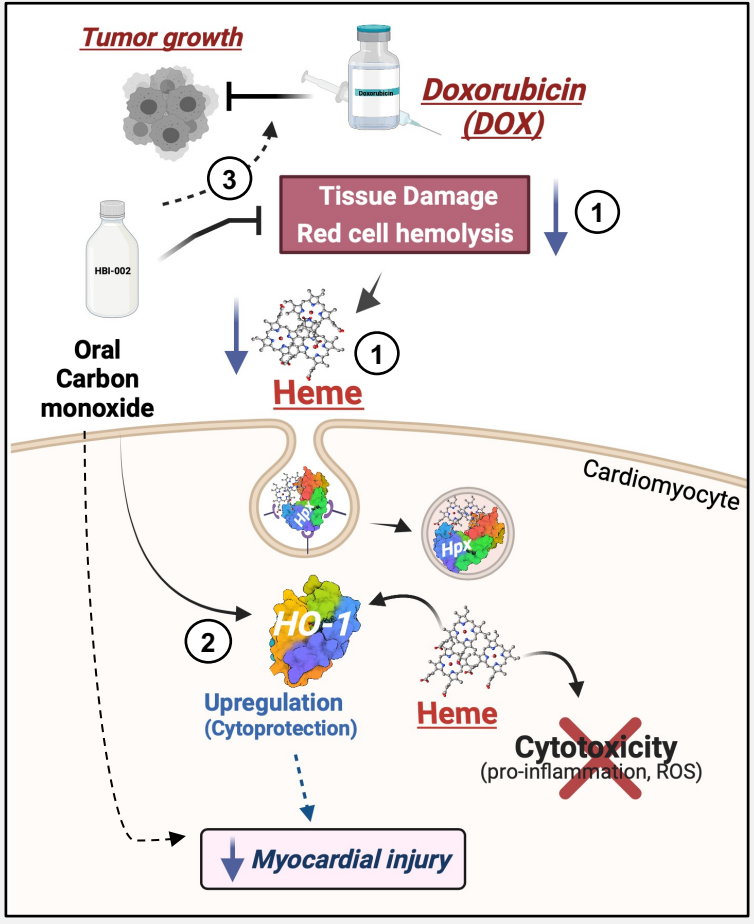
CONCLUSION

1. HBI-002 prevented acute muscle damage and serum heme release associated with DOX administration

2. HBI-002 upregulated HO-1 expression in the heart

3. HBI-002 can be administered in combination with DOX

<u>The oral CO drink HBI-002, which is designed for use in hospitals and at home,</u> may maintain the cancer therapeutic benefits of DOX and mitigate cardiac damage caused by DOX treatment.



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