Hydrogen Sulfide Attenuates Doxorubicin-induced Exacerbation of Myocardial Ischemia-Reperfusion Injury in Mice

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BACKGROUND

Cancer and cardiovascular disease are the two leading causes of death in developed nations. Paradoxically, improved long-term cancer survival has demonstrated the risk of adverse cardiac effects from cancer treatment itself. Doxorubicin (DOX) is the most utilized anthracycline and is used to treat many types of tumors. Unfortunately, the utility of this drug is limited by its side effects on the cardiovascular system.

Acute Myocardial Infarction (AMI) and DOX-mediated cardiotoxicity share overlapping cardiotoxic mechanisms, such as oxidative stress and cellular damage, leading to loss of heart function. In preclinical models of AMI, overexpression of Hydrogen Sulfide (H_2S)-producing enzymes or exogenous administration of H_2S by way of donors lowered infarct size and preserved cardiac function. H_2S also exerts antioxidant activity by maintaining mitochondrial integrity, increasing nitric oxide synthase activity, increasing Akt activation, and triggering the opening of K_{ATP} channels. H_2S treatment also inhibits NLRP3-dependent inflammation in a mouse model of AMI. Due to the negative impact of chemotherapy on the heart, the hypothesis of this study is to determine if cancer survivors are more susceptible to experiencing myocardial damage second to myocardial infarction (AMI) and if a therapy based on H_2S can mitigate these adverse conditions.

Methods

In-vitro experimental AMI: AC16 (Human proliferating Cardiomyocytes) were used to model cardiac tissue. AC16 were treated with DOX 1μM for 24 h, followed by 24 h of washout. AC16 cells were then subjected to simulated ischemia/reoxygenation (SI/RO) for 8 hours by replacing DMEM medium with an "ischemia buffer" (118 mM NaCl, 24 mM $NaHCO_3$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 20 mM sodium lactate, 16 mM KCl, pH adjusted to 6.2) and exposed to 5% CO_2 95% N_2 atmosphere in a gas chamber at 37°C. During 4-hour reperfusion, cells were treated with 50μM of GYY 4137, a slow-releasing H_2S donor. MTT assay was used to determine cell death.

In-vivo experimental AMI: DOX was delivered in C56BL mice i.p., 4 doses of 5 mg/kg every 3 days for a cumulative dose of 20mg/kg. Mice were then randomized to control diet or H_2S donor SG1002-enriched chow to achieve a weekly 40 mg/kg/day dosage. After 9 weeks of SG1002 treatment, mice underwent transient ligation of the proximal left coronary artery for 30 min, followed by 24 h of reperfusion. Triphenyl tetrazolium chloride (TTC) staining was completed after euthanasia to measure infarct size. The heart was then frozen and cut, and the infarcted tissue, the risk zone, and the whole left ventricle (LV) were determined by computer morphometry.

Results

In-vitro experimental AMI: Experimental concentration for DOX and H_2S donor, GYY4137 were empirically determined by dose-response curve in normoxic conditions (Figure 1). Pre-treatment with DOX 1μM, before SI/RO, led to an additional 60% increase in myocardial damage compared to vehicle-treated cells (87 ± 0.5% vs 27 ± 7.5%, respectively). Concomitant administration of GYY 4137 during DOX rescued AC16 cell damage by 17% (70.1 ± 0.9%) compared to DOX-only-treated cells (Figure 2).

In-vivo experimental AMI: DOX-treated mice exhibited a 15% increase in infarct size compared to vehicle-treated mice (55.5 ± 5.3% vs. 40.6 ± 2.5, respectively) (Figure 3). H_2S donor SG1002-treated mice demonstrated a 17% reduced infarct size (38.4 ± 5.6%).

Post chemotherapy, **cancer survivors are at higher risk** of greater ischemic damage during a myocardial infarction.

H_2S donors can **mitigate doxorubicin's cardiotoxic effects** and thus present as **viable therapeutic** option to **protect cancer survivors from chemotherapy-induced cardiac risk.**

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Conclusions

H_2S donors (SG1002 and GYY 4137) decrease cell death in C56BL mouse models and AC16 cardiomyocytes following doxorubicin administration. During simulated AMI, SG1002 administration displayed a downward trend in infarct size compared to mice treated with doxorubicin alone. Similarly, AC16 cardiomyocytes given doxorubicin and GYY 4137 prior to simulated AMI exhibited less cell death than the cells that received doxorubicin only.

FIGURE 1

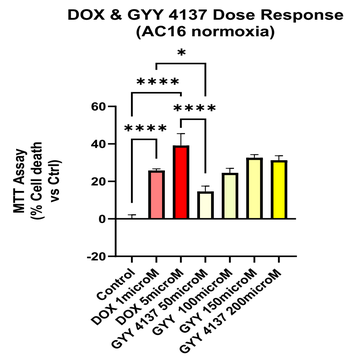


FIGURE 1. Dose Curve Response for DOX and GYY 4137. Several concentrations of DOX and GYY4137 were tested in normoxia to identify the proper experimental settings. MTT assay was used to determine cell death. Data are expressed as Mean ± SEM. *P<0.05, ****P<0.0001

FIGURE 2

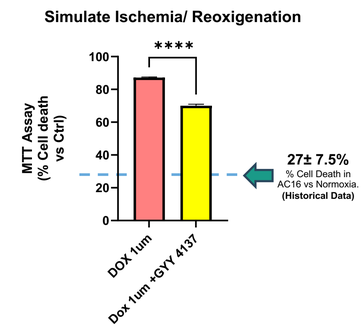
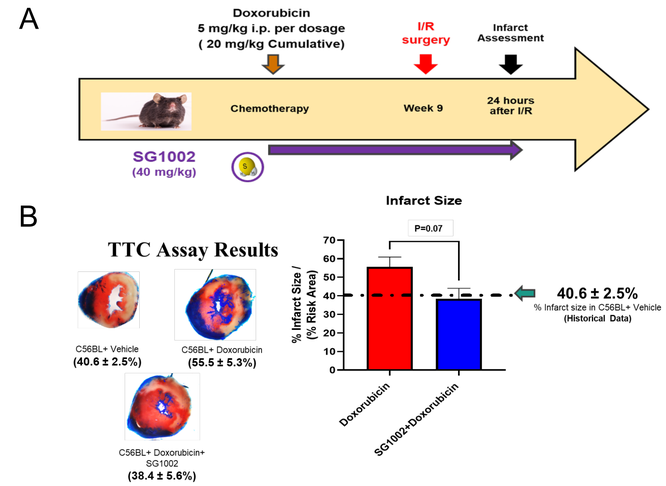


FIGURE 2. AC16 Simulated Ischemia Reperfusion with DOX and DOX + GYY 4137. Dox 1μM and Dox 1μM + GYY 5137 50μM treated AC16 cardiomyocytes were tested under ischemia/reperfusion. MTT assay was used to determine cell death. Data are expressed as Mean ± SEM. ****P<0.0001

FIGURE 3



AMI of C56BL mouse models with DOX and DOX + SG1002. A) Schematic representation of the study design in-vivo. B) Nine weeks after DOX treatment, mice with normal diet and SG1002-enriched chow diet underwent AMI surgery. TTC staining was used to determine infarct size and risk area. Data are expressed as Mean ± SEM.

Disclosures

Dr. Salloum serves on the Advisory Board for NovaMedix, LLC. All other authors declared no conflict of interest.

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