

# Effect of H<sub>2</sub>S on Branched-Chain Amino Acid Accumulation in Ventricular Cardiomyocytes following Doxorubicin Administration

Zachary Schwehr\*, Elisa Palladino†, Fadi Salloum†

\*VCU Medical Science Internship Program 2023

†Virginia Commonwealth University, School of Medicine



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## Abstract

Branched-chain amino acids (BCAA) – isoleucine, leucine, and valine — play an important role in metabolic homeostasis through nutrient signaling. At high circulation concentrations, they have been linked to various metabolic disorders and cardiovascular diseases, including heart failure. As a result, defects in BCAA catabolism may potentially be a viable therapeutic target for heart failure. Doxorubicin (DOX) is a chemotherapy drug that causes dose-dependent cardiotoxicity that results in left ventricle ejection fraction (LVEF) decline and cardiac fibrosis. Hydrogen sulfide (H<sub>2</sub>S) donors have been shown to preserve LVEF in animal models of DOX cardiotoxicity. In this study we first confirmed the cytotoxic effects of DOX on cardiomyocytes using an MTT assay. We then explored the effects of DOX on BCAA accumulation, followed by the protective effects of GYY4137, a slow-releasing H<sub>2</sub>S donor in human ventricular cardiomyocytes (AC16) after DOX administration. AC16 cells were exposed to either the vehicle (saline), DOX (1 μM), or DOX (5 μM) and incubated for 24 hours. BCAA accumulation was measured using a BCAA assay kit in a 96-well plate. DOX (5 μM) significantly increased BCAA accumulation in AC16 cells relative to those treated without DOX. Based on the results, DOX impairs BCAA catabolism resulting in increased BCAA accumulation. The effects of H<sub>2</sub>S will be explored in future experimentation following DOX by treating AC16 cells with GYY4137 at concentrations of 25, 50, or 75 μM. Additionally, mass spectrometry will be implemented for more precise BCAA accumulation measurements.

## Introduction

### • Branched-chain amino acids (BCAA) - isoleucine, leucine and valine

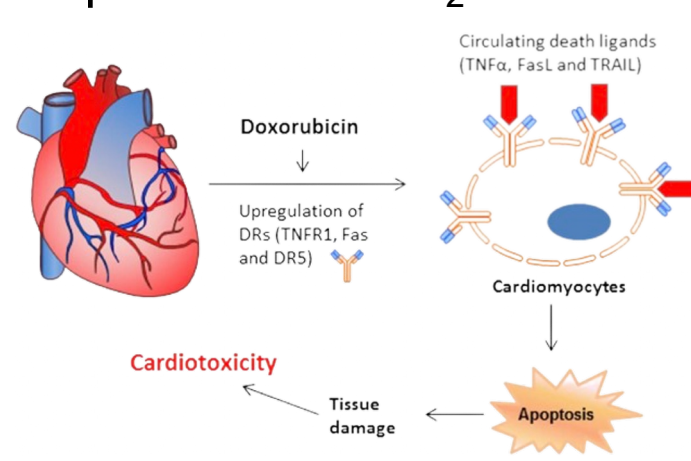
- Linked to insulin resistance, type 2 diabetes, and cardiovascular disease
- Restoration of BCAA catabolism reversed cardiac dysfunction in mice following pressure overload-induced heart failure

### • Doxorubicin (DOX) - chemotherapy drug that induces cardiotoxicity

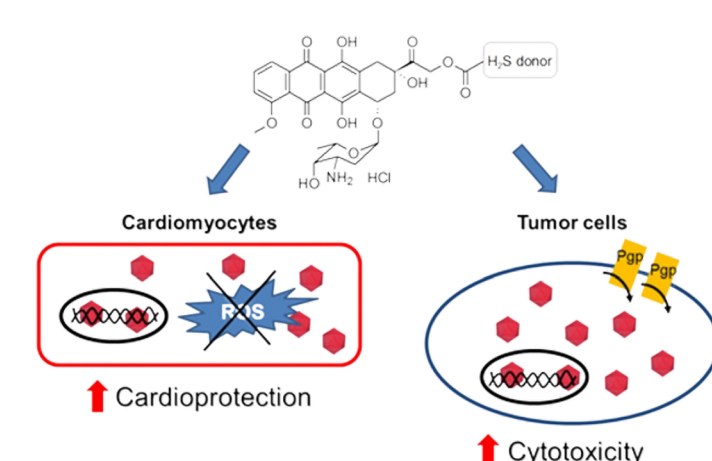
- Induction of cardiomyocyte apoptosis, which may contribute to cardiomyopathy
- Causes decline in left ventricle ejection fraction (LVEF) and fibrosis

### • Hydrogen sulfide (H<sub>2</sub>S) donor

- Causes antiapoptotic pathway activation and anti-inflammatory effects
- H<sub>2</sub>S donors have been shown to have cardioprotective properties against anthracyclines
- GYY4137 is a very slow-releasing H<sub>2</sub>S donor that mimics the physiological production of H<sub>2</sub>S



<https://doi.org/10.1038/srep44735>

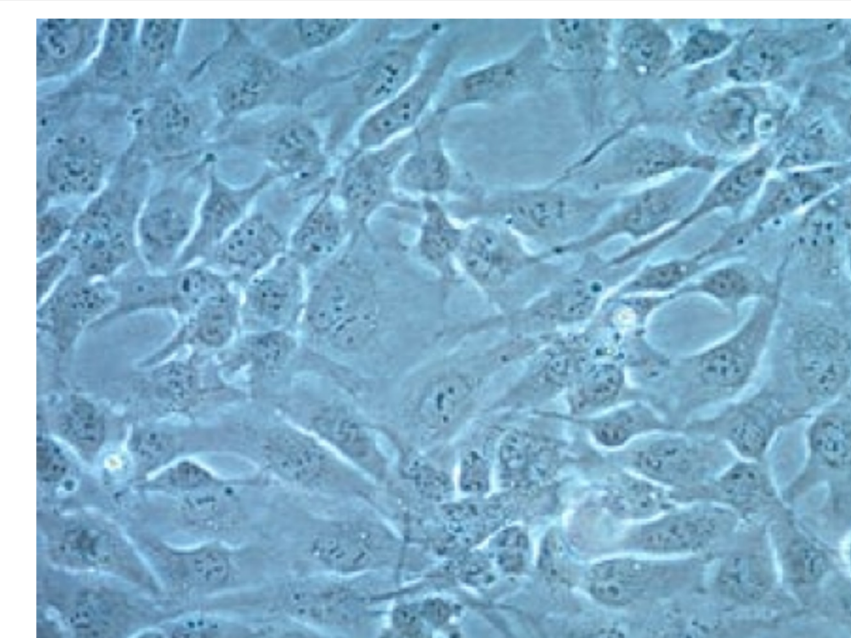


<https://doi.org/10.1021/acs.jmedchem.6b00184>

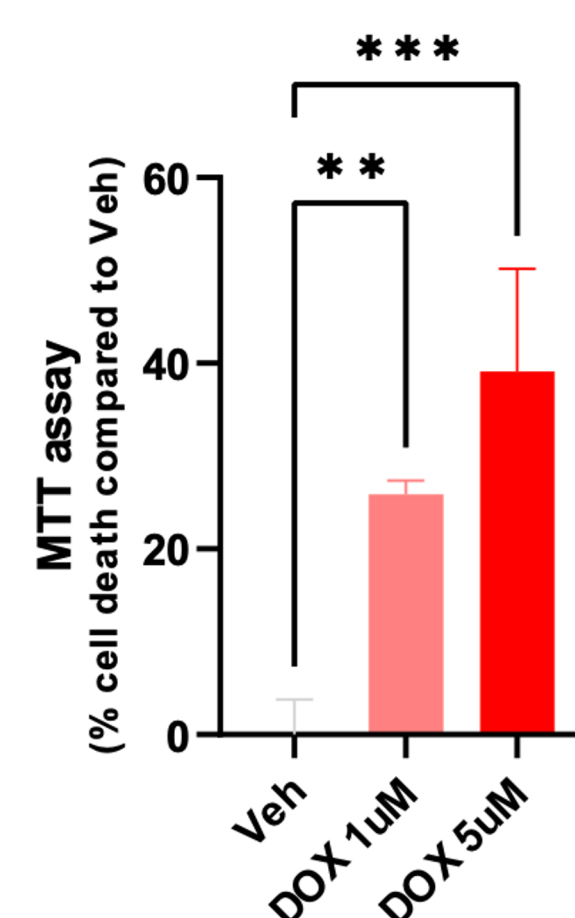
## Methods and Materials / Results

Human ventricular cardiomyocytes (AC16) were cultured in T75 flasks and incubated at 37 °C with 5% CO<sub>2</sub>

Figure 1: Depiction of the AC16 cells during cell culture

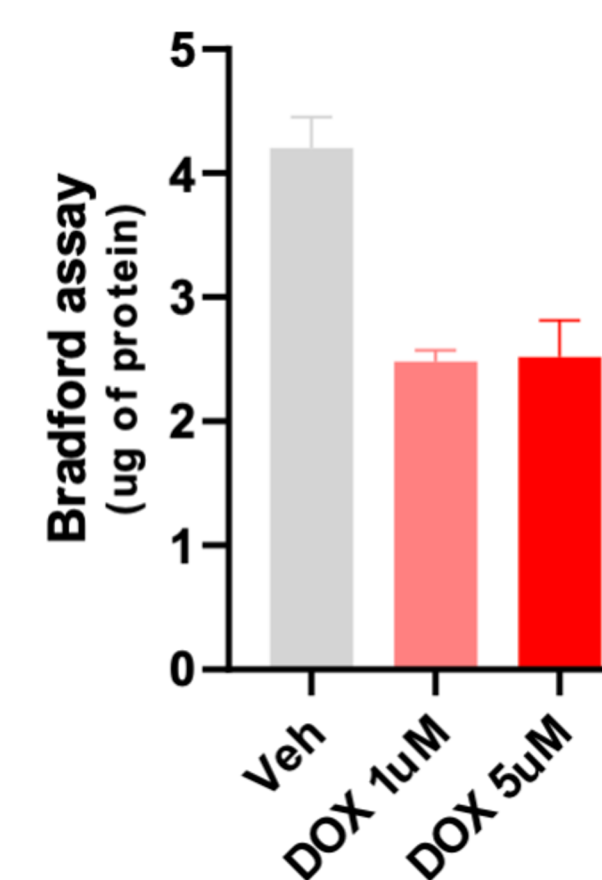


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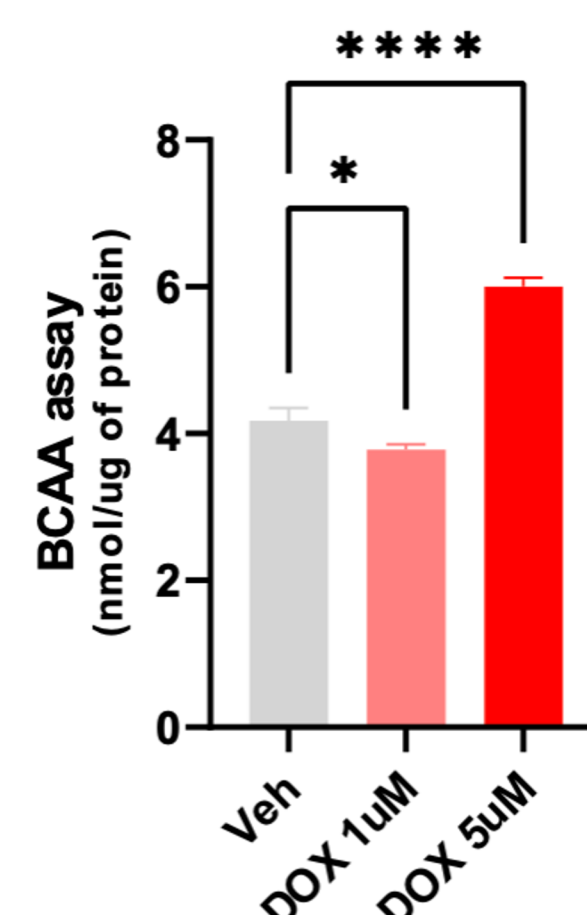
MTT assay measures cellular metabolic activity by measuring the reduction of MTT salt to formazan, which creates a colored solution ( $\lambda_{max}$  = 500-600 nm) when dissolved

Figure 2: Cell death (%) of AC16 cells after DOX. DOX decreases cell viability. Data is expressed as the mean  $\pm$  standard error. \*P<0.05; \*\*P<0.01; \*\*\*P<0.005; \*\*\*\*P<0.001



Bradford Protein Assay: Bradford reagent binds to proteins leading to the color change of a dye from 470 to 595 nm

Figure 3:  $\mu$ g protein in AC16 cells. The amount of protein reflects the number of cells in the sample. Data is expressed as mean  $\pm$  standard error.



BCAA Assay: Enzyme oxidatively deaminated BCAA, producing NADH, leading to the production of a colored product ( $\lambda_{max}$  = 450 nm)

Figure 4: BCAA accumulation nmol /  $\mu$ g protein in AC16 cells. DOX impairs BCAA catabolism after 24 hours. Data is expressed as mean  $\pm$  standard error.

\*P<0.05; \*\*P<0.01; \*\*\*P<0.005; \*\*\*\*P<0.001

## Conclusions

- Statistical analysis between the vehicle and Doxorubicin (5 μM) shows a significant increase in BCAA accumulation in AC16 cells
- These results confirm our hypothesis that Doxorubicin treatment causes BCAA accumulation, which contributes to the cardiotoxic effects of Doxorubicin treatment on the heart

## Future Directions

- Determine if H<sub>2</sub>S donors, GYY4137, attenuates DOX-induced BCAA catabolism impairment
- Employing mass spectrometry to measure BCAA accumulation instead of BCAA Assay Kit
- Perform experimentation on heart tissue samples to determine the impact of DOX and H<sub>2</sub>S on BCAA catabolism at a larger scale

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## Disclosures

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