4-23-2021

Effects of Doxorubicin on Cardiac Fibroblasts and the Extracellular Matrix

Tyler Lantz
Boise State University

Conner Patricelli
Boise State University

Steve Nick
Boise State University

Matthew Turner
Boise State University

Julia Oxford
Boise State University

See next page for additional authors

Authors

Tyler Lantz, Conner Patricelli, Steve Nick, Matthew Turner, Julia Oxford, and Xinzhu Pu

Effects of Doxorubicin on Cardiac Fibroblasts and the Extracellular Matrix

Tyler Lantz[1], Conner Patricelli[1], Steve Nick[2], Matthew Turner[1], Julia Oxford[1], Xinzhu Pu[1]

[1]Biomolecular Research Center, Boise State University, Boise, ID

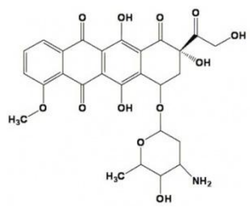
[2]Department of Chemistry and Biochemistry, Boise State University, Boise, ID

Introduction

Cardiotoxicity has been associated with various types of chemotherapeutic drugs contributing to a plethora of cardiac insults and is a significant side effect when treating cancer. Many highly effective anticancer drugs are severely dose dependent, and at higher doses can lead to: cardiac arrhythmias, hypertension, and lethal cardiomyopathy. A well known example of this cardiotoxic side effect is Doxorubicin, a common chemotherapeutic used to treat cancers of the breast, ovary, bladder, and thyroid. Extensive research has shown that high doses of doxorubicin detrimentally alters the normal function of cardiac fibroblasts and cardiomyocytes. In contrast to the extensive research on the toxic effects of chemotherapeutics like doxorubicin in cardiomyocytes, little is known on the effects in cardiac fibroblasts and mechanisms of these drugs on the cardiac extracellular matrix (cECM). We show that doxorubicin has a direct impact on cardiac fibroblasts and in turn the function of the cECM, indicating that the cECM plays an important role in cardiac toxicity induced by doxorubicin.

Doxorubicin

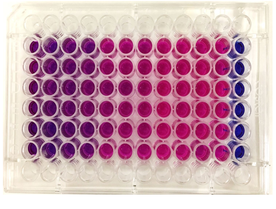
- DOX is a member of a family of highly effective chemotherapeutic drugs called anthracyclines
- Anthracyclines are isolated from the bacterial source *Streptomyces peucetius var. caesius*
- DOX has three mechanisms of cytotoxicity, which also affect non-cancerous cells:
  - Inhibition of topoisomerase II
  - Production of reactive oxygen species
  - DNA intercalation

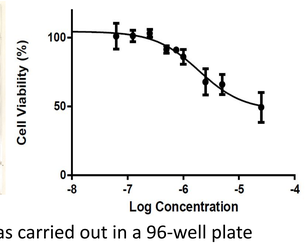


Structure of Doxorubicin

Cytotoxicity Test

- Primary cardiac fibroblasts (CFBs) isolated from BALB/c mice were exposed to various concentrations of DOX for 24 hours.
- Resazurin was added three hours prior to the measurement.
- 1 µM DOX yielded the cell viability of 70-80%. This concentration was used in all the subsequent experiments.

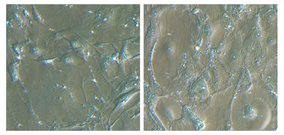




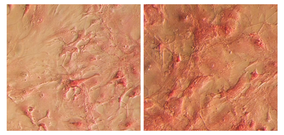
The cytotoxicity assay (left) was carried out in a 96-well plate with concentrations of DOX ranging from high to low. The pink wells have higher cell viability. 1 µM of DOX yielded 70-80% and IC_{50} was approximately 25 µM.

ECM Staining

CFBs grown in 6-well plates were exposed to 1µM DOX for 24-hours. Culture media were then replaced with media free of DOX. After five days, CFBs were stained with Alcian blue for glycoproteins and picrosirius red for collagens.

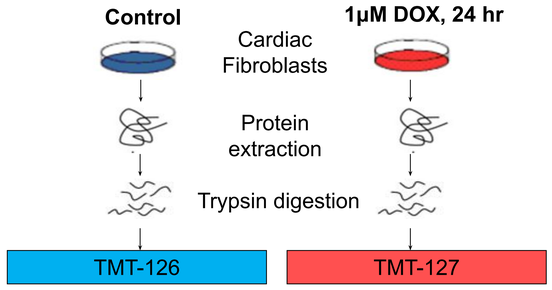


Alcian blue staining of CFB control (left) and DOX-treated (right)



Picrosirius red staining of CFB control (left) and DOX-treated (right)

Duplex TMT Assay



Combine labelled peptides

↓

High pH reverse phase fractionation

↓

Low pH reverse phase LC

↓

Tandem MS

↓

Protein identification and quantification

↓

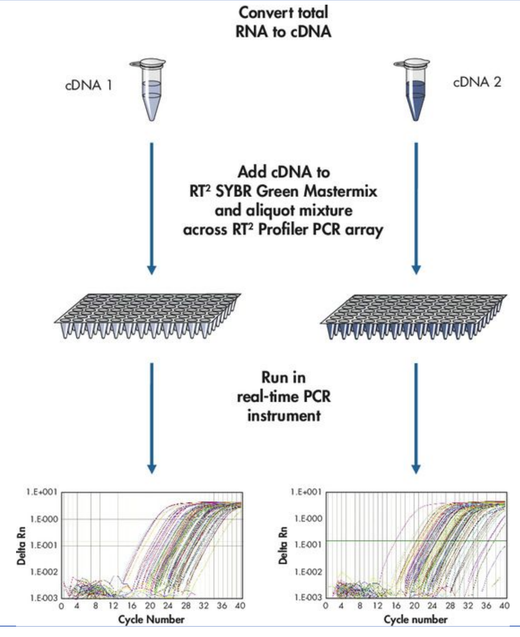
Differentially expressed protein list

- Velos Pro Dual-Pressure Linear Ion Trap Mass Spectrometer (Thermo Fisher Scientific)
- C18 reverse phase nano column: 0.075 x 100 mm, 3µm, 120Å
- HCD fragmentation with 45% normalized collision energy
- Proteome Discover 2.2 (Thermo Fisher Scientific)
- Sequest HT search engine
- Sequence database: UniProtKB/Swiss-Prot protein database for mouse (obtained on December 10, 2018)

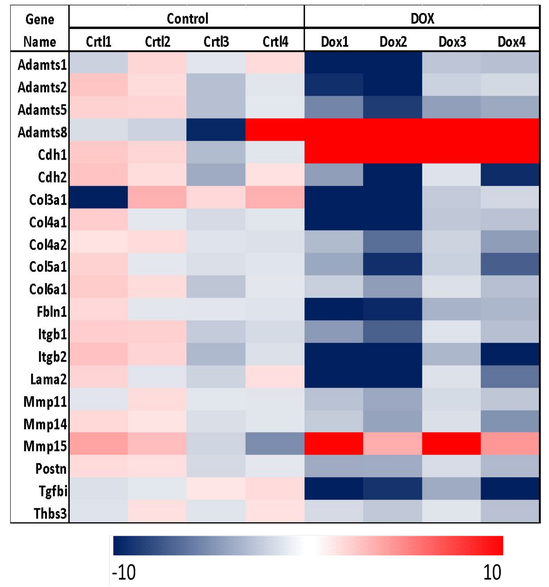
Differentially expressed protein list

Protein	Fold of Change
Endothelial protein C receptor	2.98↑
Isoform 2 of Intercellular adhesion molecule 1	2.46↑
Aldehyde dehydrogenase family 1 member A3	2.43↑
Arachidonate 5-lipoxygenase	1.96 ↑
L-lactate dehydrogenase B chain	1.95↑
BLOC-1-related complex subunit 7	1.85↑
Protein NDRG1	1.83↑
Serine protease inhibitor A3G	1.81↑
Antizyme inhibitor 1	1.80↑
Syndecan-4	2.52↓
Denticleless protein homolog	2.42↓
Profilin-3	1.98↓
Myb-binding protein 1A	1.97↓
Potassium voltage-gated channel subfamily E member 4	1.90↓
Thrombospondin-1	1.89↓
Transmembrane	1.83↓
Ribosomal L1 domain-containing protein 1	1.81↓
Thrombospondin-2	1.81↓

Quantitative PCR Analysis



Expression of Extracellular Matrix & Adhesion Genes



Future Work

Future aim is to examine the direct intercellular signaling between doxorubicin treated fibroblasts and cardiac myocytes. Primary cardiac fibroblasts and cardiomyocytes isolated from BALB/c mice

- Co-culture of Dox treated with fibroblasts and cardiomyocytes
- Endpoints
  - Global gene expression changes in myocytes: RNA-seq
  - Cardiomyocyte functional assay: Fluorescent imaging using calcium indicator

Acknowledgements

We acknowledge support from the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408, P20GM109095, and *1C06RR020533*. We also acknowledge support from The Biomolecular Research Center at Boise State, BSU-Biomolecular Research Center, RRID:SCR_019174, with funding from the National Science Foundation, Grants #0619793 and #0923535; the M. J. Murdock Charitable Trust; Lori and Duane Stueckle, and the Idaho State Board of Education. We would especially like to acknowledge the support from the Higher Education Research Fellowship Program (HERC)