Integration of Multiple Cardiac Parameters to Predict Drug-Induced Cardiac Toxicity

Kim A. Henderson[1], R. Brandon Borders[1], John B. Ross[1], Amir Jalil[2] , and Brian M. Roche[1], [1]*Battelle, Columbus, OH,* [2]*Ohio State University, Columbus, OH*

ABSTRACT #0021
POSTER #0084

ABSTRACT

Cardiac toxicity, manifested as compromised contractility or ischemic heart disease, comprises 26.9% of post-approval drug failures. To address issues of attrition, safety and cost of drug development, there is a need to engage in more rigorous preclinical cardiac safety testing. The use of a multi-scale approach for assessing cardiac toxicity may detect signs and indicate mechanisms of toxicity that would otherwise go unnoticed by using traditional measures of cardiovascular function. The purpose of this research was to utilize the Langendorff isolated heart model to assess integrated parameters in response to drugs with known/unknown mechanisms of toxicity. We integrated ECG, contractility, energetic, and biomarker responses to assess the effects of FCCP, verapamil, doxorubicin, doxorubicin-ol, sorafanib and sunitinib. Rat hearts were removed, arrested, and perfused with Modified Henseliet Krebs. Left ventricular pressure was monitored via a fluid-filled balloon, while electrodes on the heart measured ECGs. NMR spectroscopy of ^{31}P found in ATP, phosphocreatine, and inorganic phosphate was performed by inserting the heart into an 11.7T NMR. We recorded a baseline period followed by 60 min of drug exposure and 20 min of 0.1µM isoproterenol with drug to assess energy reserve. Heart effluent was collected at the end of drug exposure for analysis of biomarkers. Combining multiple endpoints allowed for detection of acute injury as indicated by biomarker and energetic responses, even when contractility appeared to be normal or enhanced, as for doxorubicin. We have shown that integrating cardiac molecular responses with traditional functional responses allows for greater insight into predicting cardiac toxicity.

BACKGROUND

Challenges facing drug development

- Cardiac toxicity: 26.9% of drugs removed after approval are due to issues with contractility or ischemic heart disease
- Latent toxicity: Several chemotherapies cause heart failure manifested as long as 10 years after treatment, difficult to detect in typical preclinical and clinical testing

Urgent need for more predictive cardiac safety testing

- Must be sensitive, predictive, and cost effective

Integrated, multi-scale approach to cardiac safety assessments earlier in drug discovery

- Better inform decisions and optimize lead candidates
- Reduce overall costs
- Reduce animal usage by utilizing and integrating multiple data end points

Goal: Determine functional and molecular profile (contractility, ECG, high energy phospogens) of cardiac effecter drugs with known/unknown mechanisms of action using FCCP (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone), Verapamil, Doxorubicin, and Sorafanib as test articles

METHODS

***Ex-vivo* (Langendorff isolated heart)**

- Sprague Dawley rats anesthetized with Na-Pentobarbital (80 mg/kg). Hearts removed and placed in cardioplegic solution prior to perfusion with Modified Krebs-Henseliet solution (118 NaCl, 1.18 KH_2PO_4, 25 $NaHCO_3$, 5 KCl, 1.17 $MgSO_4$, 11 glucose, 2 NaPyruvate, 1.8 $CaCl_2$ mM) in a Langendorff system (Emka, France).
- Water-filled balloon inserted into left ventricle to measure left ventricular pressure (LVP) parameters ($dPdt_{max}$ and $dPdt_{min}$, developed pressure, end diastolic pressure), coronary flow rates, heart rate, and ECG waves are simultaneously measured

Protein Biomarkers

- Effluent is collected from the heart at end of dosing, concentrated using Millipore Centricon tubes, and analyzed for the appearance of protein biomarkers TnT, TnI, TNFα, IL6, and BNP using Milliplex antibody assay (Millipore, MA)

Cardiac Energetics (^{31}P analysis using NMR)

- Isolated Langendorff perfused heart is mounted inside an 11.7T wide bore magnet. ATP, phosphocreatine (PCr), and inorganic phosphate (Pi) content is assessed by acquiring spectra of ^{31}P at 202.4 MHz every 4 minutes

Drug Exposure

- Dose escalation: 20 min baseline, 20 min each dose escalation
- NMR energetics profile: 20 min baseline, 60 min drug, 20 min drug + 0.1µM isoproteranol (iso)

TECHNICAL APPROACH

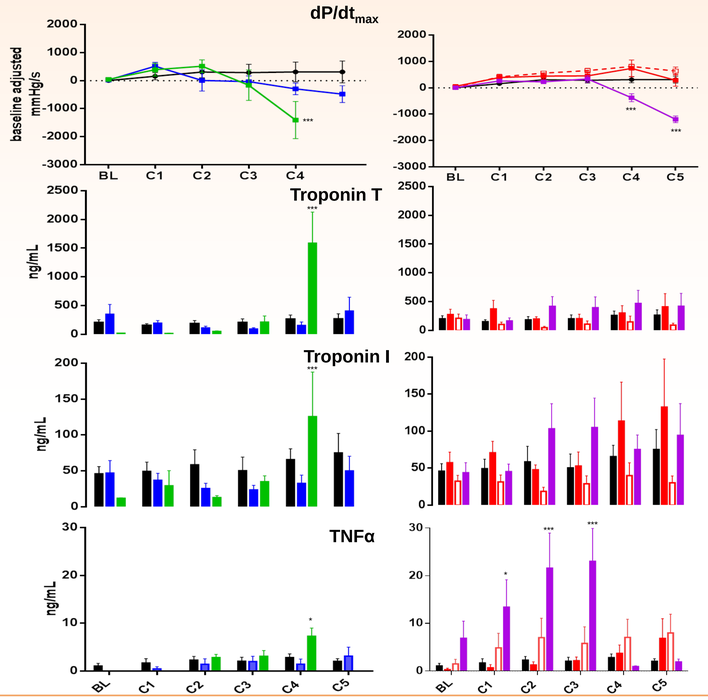
Drug Effects on Contractility and Appearance of Biomarkers in Cardiac Effluent

1. Dosing Scheme

Baseline 20 min	Drug: escalating concentrations 20 min each

Effluent sample End of 20 min — Effluent sample End of 20 min

	C1	C2	C3	C4	C5	[M]
Control (n=9)						
Verapamil (n=5)	10^{-9}	10^{-8}	$3x10^{-8}$	$6x10^{-8}$	10^{-7}	
FCCP (n=4)	10^{-9}	10^{-8}	$3x10^{-8}$	$6x10^{-8}$		
Doxorubicin (n=6)	10^{-9}	10^{-8}	10^{-7}	**10^{-6}**	10^{-5}	
Doxorubicin-ol (n=6)	10^{-9}	10^{-8}	**10^{-7}**	$3x10^{-7}$	10^{-6}	
Sorafanib(n=5)	10^{-7}	10^{-6}	**$3x10^{-6}$**	$6x10^{-6}$	10^{-5}	

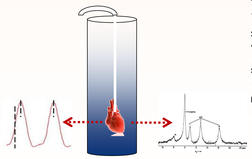


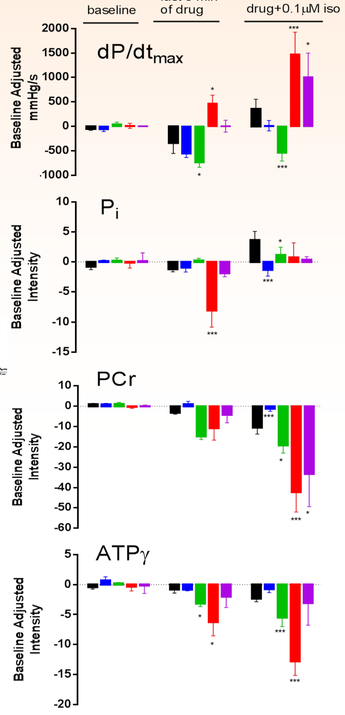
Drug Effects on Heart Contractility and Energetics Reveal Signs of Toxicity

2. Dosing Scheme

Baseline 20 min	Drug 60 min	Drug + 0.1µM Iso 20 min

	[M]
Control (n=6)	
Verapamil (n=5)	10^{-7}
FCCP (n=4)	$3x10^{-8}$
Doxorubicin (n=5)	10^{-6}
Sorafanib(n=3)	$3x10^{-6}$





CONCLUSIONS

Results Summary

Treatment	Contractility		ECG Interval	Energetics		Stress Test		Cell Stress	Cell Death
	Drug	Stress Test		Pi	PCr	Pi	PCr		
Verapamil	⇩	⇧	?	⇩	—	—	—	—	—
*FCCP	* ⇩	—	?	—	* ⇩	* ⇧		*** ⇧	*** ⇧
*Doxorubicin	* ⇧	*** ⇧	—	*** ⇩	⇩	** ⇧	*** ⇩	⇧	—
*Doxo-ol	⇧		—	?	?	?	?	⇧	—
*Sorafanib	—	* ⇧	?	⇩	⇩	⇧	* ⇩	*** ⇧	—
Control	⇩	⇧	—	—	⇩	⇧	⇩	—	—

Monitoring changes in contractility alone does not always predict toxicity

- Exemplified by anthracyclins and tyrosine kinase inhibitors

Combining multi-scale endpoints can provide better insight into potential for toxicity and affected pathways

- Contractility with drug:
  - Compared to control, acute decreases were seen in FCCP and verapamil, with slight increases with anthracyclines
- Energetics:
  - FCCP decreased PCr
  - Doxorubicin decreased Pi
- Stress test with drug:
  - Contractility dramatically increases with doxorubicin and sorafanib
  - Energetics dramatically reduced with doxorubicin and sorafanib
  - Biomarkers of stress and injury
    - Cell death only seen with FCCP
    - Significant cell stress (as indicated by TNFα cytokine response) seen in FCCP and sorafanib
      - Doxorubicin and Dox-ol group had 1 heart in each that responded strongly
    - Combining energetics responses during drug and during stress test and increases in TNFα were more predictive of known toxicants than contractility alone

Next Steps

- Further analysis into drug induced cardiac immune response
  - Additional cytokines
- Further investigation into cardiac reserve
- In vivo translation