Whole Heart Energetics and Stress Test as an Indicator of Drug Induced Cardiac Toxicity

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ABSTRACT

Cardiac toxicity, manifested as compromised contractility or ischemic heart disease, comprises 27% of post-approval drug failures. The heart has a high demand for a constant energy supply which can be affected by many sources of stress and thus may be a good indicator of potential toxicity. The purpose of this research was to utilize the ex vivo heart model to assess contractility and whole heart energetics in response to drugs with known/unknown mechanisms of toxicity. We used FCCP and venamycin as positive and negative controls and doxorubicin (dxo), doxorubicin-ol, sorafenib as chemotherapeutics associated with latent toxicity. In vivo hearts were removed, perfused with Modified Krebs-Henseleit and LVP was monitored via a fluid-filled balloon. The perfused heart was inserted into an 11.7 T MRI magnet. Whole heart phosphoglycerate content was assessed before and after 60 min of drug exposure and then during 20 min of 0.1 µM isoproterenol (iso) with drug to assess energy reserve. Control heart contractility and energetics were stable throughout the experiment until the isoc challenge, where contractility increased as expected and PCl and ATP decreased and PI increased. FCCP treated hearts showed a decline in contractility and PCl and reduced reserve during the isoc challenge. Venamycin treated hearts did not change in energetics during treatment or during the isoc challenge. Dxo increased contractility, while the other chemotherapeutics showed little change in contractility during drug treatment. Dxo treated hearts demonstrated a drop in Pi, PCl, and ATP. In addition, during the isoc challenge contractility increased compared to control and PCl decreased. Dxo and sorafenib may be entrapping the part of the isoe-agonal pathway, causing a more pronounced response to iso and thus resulting in an increased work load to the heart. This may be a possible pathway to investigate as a mechanism for latent toxicity, as well as an early indicator of potential drug induced cardiac issues after treatment.

CONCLUSIONS

Viability of NMR to Detect Drug Induced Changes in Cardiac Energetics

We demonstrated that NMR can be used to detect changes in high energy phosphates using FCCP (30µM) (micromolar unconcentrated) as a positive control. A reduced PCl and ATP were observed. Venamycin (10µM) (a L-type Ca-channel blocker) as a negative control reduced PCl by >90% and effects on energetics. Doxorubicin (0.1µM) was different from control in function or energetics. Sorafenib (1 µM) and Erlotinib (1 µM) did not affect PCl or ATP during 60 minutes of perfusion.

Viability of Stress Test to Ascertain Energy Reserve

We used the S p-adrenergic isoprenaline (0.1 µM) to increase cardiac work in order to determine heart energetic reserves after 60 minutes of drug treatment.

Assumptions

- The heart must have adequate levels of PCl and the ability to produce ATP and utilize PCl to respond to isoproterenol.
- If energy levels are affected by drug, then the heart may not respond to iso and may demonstrate higher reduction in PCl and ATP during treatment
- We found that hearts treated with FCCP responded as expected (compared to control) with reduced contractility and greater reductions in PCl and particularly ATP.

- Dxo and Sorafenib are toxic and sorafenib may fit the model.
- Hearts pretreated with doxorubicin followed by isoprenaline had enhanced contractility and decreased reductions in PCl and ATP.
- Hearts pretreated with sorafenib and erlotinib followed by isoprenaline also had enhanced contractility compared to controls and a greater reduction in PCl but not ATP.
- This suggests that sorafenib, sorafenib, and erlotinib all enhance the pathway activated by S p-adrenergic isoproterenol, possibly causing an additive effect due to common mechanisms.
- Activation of PKA, AMP, CAMK which could have increased effects on Ca handling and/or removal.
- Dissociation but not dox may affect energetics and enhanced S p-adrenergic response whereas sorafenib and erlotinib enhance energetic based on stability of ATP during isoprenaline.
- Combined NMR isolated heart is a powerful tool to assess cardiac function and energetics as a biomarker of drug induced toxicity. Due to the complex mechanisms involved in S p-adrenergic signaling and in chemotherapeutic actions on the heart, it may be better to assess cardiac energy reserve in a non-murine such as external pacing.

METHODS

Drug Exposure
- Acute Ex vivo (Langendorff isolated heart) via retrograde perfusion of the coronary arteries
- All drugs were dissolved in Modified Krebs-Henseleit (MKH) solution as the vehicle: venamycin (0.1 µM) FCCP (30µM), doxorubicin (0.1µM), sorafenib (1µM), and Erlotinib (1µM).

Ex vivo (Langendorff Isolated heart):
- Sprague Dawley rats were anesthetized with Na-Pentobarbital (80 mg/kg). Hearts were removed and placed in cardioplegic solution prior to perfusion with Modified Krebs-Henseleit Solution (1:1 NaCl, 1.144 mM PO4, 25 NaHCO3, 5 KCl, 1.17 MgSO4, 11 glucose, 2 NaPyruvate, 1.6 CaCl2, Mg) in a Langendorf system (Emda, France)
- A fluid-filled balloon inflated into the left ventricle to measure left ventricular pressure (LVP) parameters (dP/dtMax and dP/dtMin, developed pressure, and diastolic pressure); perfusion pressure, heart rate are simultaneously measured Cardiac energetics (H+ analyses using NMR)
- The isolated Langendorf perfused heart was mounted inside an 11.7T wide-bore magnet, ATP, phosphocreatine (PCr), and inorganic phosphate (Pi) content is assessed by acquiring spectra of 7TP at 2024 MHz every 4 minutes.
- Drug exposure: 20 min baseline with vehicle only, 60 min-drug, 20 min drug + 0.1µM isoproterenol (iso)

REFERENCES

- Abstract #75 Poster #109