

# Cardio-Oncology: Anticancer Therapy Alters Cardiac Energetics; Potential Insight in to Cardiac Injury

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## ABSTRACT

Cancer patients today live longer compared to just several decades ago, which is a testimony to the advances in cancer diagnosis and therapy. However, undergoing cancer treatment increases the risk for developing cardiovascular disease later in life. Longer cancer survival is revealing latent drug-induced cardiac injury in an ever increasing number of cancer survivors. There is no clear understanding of how anticancer therapy stresses the heart or the cardiovascular system, but we do know the mechanisms vary depending on the type of treatment. The nonclinical assessment of chemotherapeutics, including biologics, may not have received the scrutiny needed to assess cardiovascular liabilities during drug development, as the priority of treating cancer outweighed the risk of producing drug-induced cardiotoxicity. The approach of assessing cancer therapeutics non-clinically needs to be evaluated and improved. We studied the effects of an anthracycline chemotherapeutic, doxorubicin (DOX), on hemodynamic function in combination with real-time assessment of the cardiac phosphorus spectra (<sup>31</sup>P) in isolated perfused rat hearts. DOX (1.0 μM, n=5) was perfused through isolated rat hearts for 60 min. Left ventricular pressure was measured directly by balloon and pressure transducer, ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) were measured by <sup>31</sup>P NMR spectroscopy. DOX induced positive effects on contractility and increased developed pressure at 1.0 μM, whereas ATP, PCr and Pi decreased. These initial studies support the possible use of <sup>31</sup>P NMR to detect decreases in energetics in the face of positive functional responses to an agent known to induce latent cardiotoxicity.

## INTRODUCTION

Cancer patients today live longer compared to just several decades ago; which is a testimonial to the advances in cancer diagnosis and therapy. Five-year relative survival rates for all sites (anatomical) increased from 50% to 66 % from 1975 to 2002 with prostate, melanoma and breast cancer survival topping the list (NCI, 2006). However undergoing cancer treatment or chemoprevention increases the risk for developing cardiovascular disease later in life. Longer cancer survival is revealing latent drug-induced cardiac damage in an ever increasing number of cancer survivors; no longer are childhood leukemia patients the only ones surviving cancer to develop latent drug-induced cardiotoxicity. Additionally, as cancer survivors age, they are at risk of developing cardiovascular disease. There is no clear understanding of how anticancer therapy stresses the heart or the cardiovascular system, but we do know the mechanisms vary depending upon the type of treatment. Albini et al (2009), states that cardiotoxicity can develop in a subacute, acute or chronic manner. Acutely, with abnormalities in ECG, and chronically, with clinical symptoms (i.e., left ventricular dysfunction) occurring following treatment or considerably after treatment. The nonclinical assessment of chemotherapeutics (ICH S6), including biologics (ICH S9), may not have received the scrutiny needed to assess cardiovascular liabilities during drug development, particularly chronic, as the priority of treating cancer outweighed the risk of producing drug-induced cardiotoxicity. The approach of assessing cancer therapeutics non-clinically needs to be evaluated and improved.

The objectives of this study were to determine the effects of escalating doses of doxorubicin on hemodynamic function and determine the effects of a single dose constant perfusion on hemodynamics with real-time assessment of the cardiac phosphorus spectra (<sup>31</sup>P) in isolated perfused rat hearts.

## METHODS

This study was conducted after approval of the Institutional Animal Care and Use Committee (IACUC) of Battelle and in compliance with USDA regulations.

**Heart Preparation:** Twenty-three male Sprague Dawley rats were anesthetized and hearts were quickly removed and submerged in 50mL chilled cardioplegic solution. Hearts were secured to the cannula of the emka© isolated heart apparatus via suture and then reanimated (after a 10 minute arrested state) by retrograde perfusion of modified Krebs solution at 37°C. A fluid-filled balloon attached to a pressure transducer was inserted into the left ventricle and slowly inflated to a preload of approximately 8-10 mmHg.

**Experiment A:** Fourteen hearts were equilibrated to the Krebs solution perfusion for 20 minutes and then Krebs containing 0.1% DMSO (vehicle) for an additional 20 minutes. Baseline parameters of contractility (dP/dt max and min, left-ventricular developed pressure, end diastolic pressure), heart rate (HR) and coronary flow parameters were collected for at least 20 minutes following equilibrium. Five hearts were then perfused with 5 increasing concentrations of doxorubicin in consecutive 20 minute durations and nine hearts were perfused with vehicle as time matched controls.

**Experiment B:** Whole heart <sup>31</sup>P content was assessed by inserting the perfused isolated heart (n=11), prepared as described above in heart preparation, in a proton/phosphorus dual tune volume RF coil (30 mm diameter and 50 mm length) and placed into an 11.7T wide bore vertical superconducting magnetic. <sup>31</sup>P frequency was 81.01 MHz as determined by using a positive control sample of ATP dissolved in Krebs at pH 7.4. First and second order shims were optimized using the proton signal arising from the water at 500.1 MHz. Then, <sup>31</sup>P spectra at 202.4 MHz were acquired every four minutes for 60 minutes post dose (plus 20 minutes for isoproterenol challenge). Each spectrum is the average of 480 acquisitions. Acquisitions were collected every 500 ms after the transmission of a 50 μs square RF pulse. Peak height before and after drug exposure was used to determine changes in inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine triphosphate (ATP). Hearts were perfused with vehicle (n=6) for time matched control or 1.0 μM doxorubicin for 60 minutes. After 60 minutes, all hearts were perfused with 0.1 μM isoproterenol for 20 minutes.

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## RESULTS

**Experiment A:** Heart rate slightly increased at concentrations  $\geq 0.1 \mu\text{M}$  doxorubicin, as compared to controls. Developed pressure and  $dP/dt_{\text{max}}$  appeared to increase with the low dose (0.001  $\mu\text{M}$ ) of doxorubicin and remained increased until the highest dose of 10  $\mu\text{M}$  as compared to controls. (Figure 1 A – F).

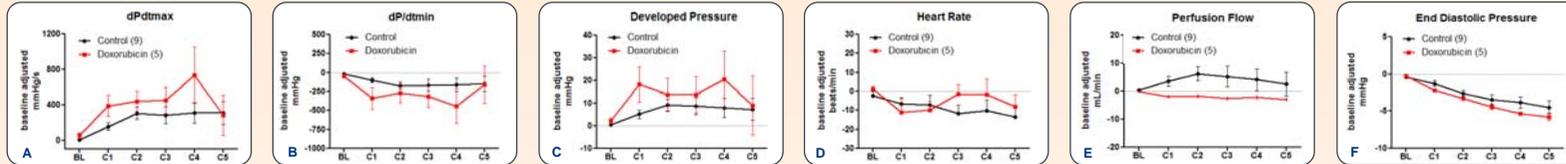


Figure 1 A – F. Functional measures of whole heart performance following dose escalation of doxorubicin from 0.0001 to 10  $\mu\text{M}$ .

**Experiment B:** Heart rate did not change and  $dP/dt_{\text{max}}$  increased, as compared to controls, over the 60 minutes of perfusion with 1  $\mu\text{M}$  doxorubicin (Figure 2 A – E). ATP, PCr and Pi all decreased when comparing control hearts to 1  $\mu\text{M}$  doxorubicin (Figure 3 A – C). Additionally, the final 5 minutes of perfusion with doxorubicin alone is shown in Figure 4 A – E. When isoproterenol (0.1  $\mu\text{M}$ ) was added to the perfusion, heart rate increased slightly more than controls and  $dP/dt_{\text{max}}$  increased when compared to the control hearts. ATP and PCr decreased more so for the doxorubicin hearts than the control hearts and Pi did not increase in the doxorubicin hearts to the magnitude of the control hearts.

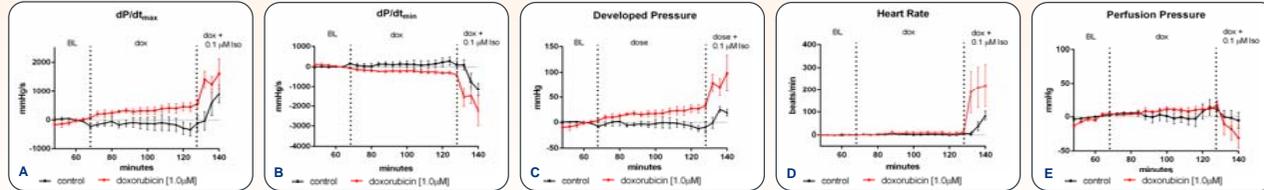


Figure 2 A – E. Functional measures of whole heart performance following constant perfusion with 1.0  $\mu\text{M}$  doxorubicin for 60 minutes followed by 20 minutes of doxorubicin and isoproterenol (0.1  $\mu\text{M}$ ). Data points are 4 minute averages.

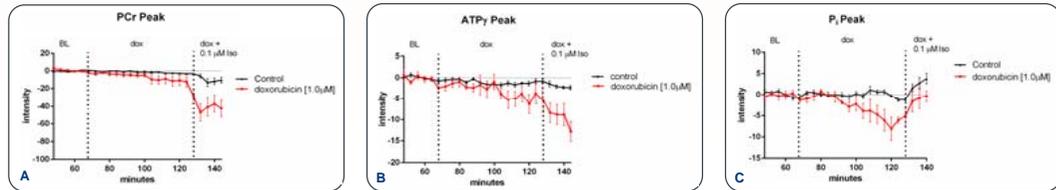


Figure 3 A – C.  $^{31}\text{P}$  NMR data for ATP, PCr and Pi following baseline, 60 minutes of doxorubicin perfusion and 20 minutes of doxorubicin and isoproterenol (0.1  $\mu\text{M}$ ). Acquisitions were averaged every 4 minutes.

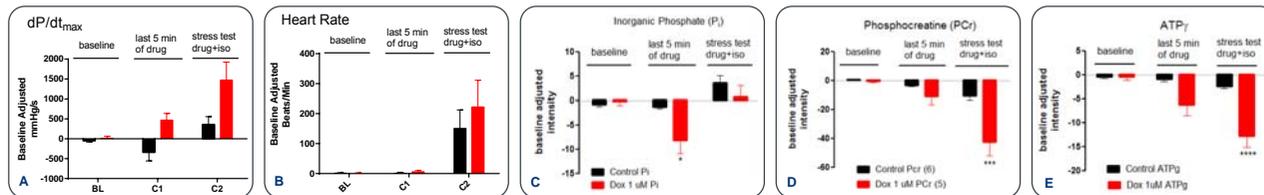


Figure 4 A – E.  $^{31}\text{P}$  NMR data,  $dP/dt_{\text{max}}$  and heart rate averaged for baseline, last 5 minutes of perfusion with doxorubicin and last 5 minutes of perfusion with doxorubicin and isoproterenol.

## DISCUSSION

The escalation dose design in Experiment A was used to establish a dose of doxorubicin that changed function but not overtly. Additionally, 1  $\mu\text{M}$  is similar to the blood concentrations in patients exposed clinically. This study also revealed that doxorubicin at lower doses, below 10  $\mu\text{M}$ , increased the strength of contraction of the left ventricle as indicated by increases in  $dP/dt_{\text{max}}$ . It appears that the 10  $\mu\text{M}$  dose started to reverse this trend.

Perfusing hearts in Experiment B while they are positioned within an NMR magnet allowed for the real-time collection of both function ( $dP/dt$ ) and energetics (ATP, PCr and Pi). The experiment was designed to determine if the energetic endpoints were altered by doxorubicin and if so, did these changes occur before the functional change and/or with greater magnitude. Additionally, would combining these assays yield additional information that alone would not. It is clear that energetics are altered, in fact, ATP, PCr and Pi all decrease in the face of increasing demand of a more forcefully contracting heart. As ATP and PCr levels decrease the inorganic phosphate should be building up in the cell, however in this experiment the inorganic phosphate is being removed. It's not until isoproterenol is added and the  $dP/dt_{\text{max}}$  increases three-fold and that Pi levels increase.

An issue with doxorubicin is the latent heart damage that occurs in up to 15% of patients treated with anthracycline drugs. The energetics shift that was demonstrated in this study may be one of the mechanisms responsible for the delayed heart damage.

## CONCLUSION

Doxorubicin, at clinical PK concentrations, increased contractility with decreases in whole heart energetics. It may be the alteration in energetics and the imbalance between functional demands and cellular response that leads to latent heart damage with doxorubicin.