JACC: CARDIOONCOLOGY
PUBLISHED BY ELSEVIER ON BEHALF OF THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION.

VOL. 5, NO. 3, 2023

EDITORIAL COMMENT

New Insight Into Cardioprotection From Anthracyclines

Still Converging on the Mitochondria*

Qun Chen, MD, PhD,[a] Giselle C. Meléndez, MD,[b,c] Edward J. Lesnefsky, MD[a,d,e,f]

Anthracycline chemotherapy remains a cornerstone of multiple cancer treatment regimens, including for lymphoma and breast cancer, although concerns for anthracycline-mediated cardiac toxicity persist.[1] Although meticulous dose adjustment has largely alleviated acute cardiac toxicity, subacute and chronic anthracycline-induced cardiac dysfunction remains a substantial clinical challenge and has spurred extensive study into the mechanisms of anthracycline-mediated cardiac toxicity. Fortunately, the potential mechanisms of cardiac toxicity appear distinct from the mechanisms of anthracycline-mediated tumor cell killing. Previously studied mechanisms of anthracycline-mediated cardiac toxicity include the enhanced production of reactive oxygen species (ROS) from mitochondria,[2] with the oxidatively sensitive mitochondrial phospholipid cardiolipin a prominent target.[3] Iron-catalyzed mechanisms enhance the ROS-mediated injury[4] and lead to iron-mediated cardiomyocyte death.[5,6] Alternatively, anthracyclines inhibit topoisomerase 2α, a key enzyme for DNA repair, leading to tumor toxicity. Unfortunately, inhibition of topoisomerase 2β in the mitochondria leads to mitochondrial damage, increasing cardiac toxicity, potentially via greater mitochondrial production of ROS.[7,8] Dexrazoxane was developed as a cell-permeable iron chelator to address potential iron-enhanced ROS-mediated cardiac toxicity. Intriguingly, this compound also exhibits partial inhibition of topoisomerase 2β,[1,7,9] potentially consolidating mitochondria-based cardiac protection. However, clinical intervention with dexrazoxane has yielded only modest protection, mainly in the pediatric population.[7,9] Thus, additional mechanistic insight into anthracycline-mediated cardiac toxicity is needed.

Cheng and his laboratory have shown that cyclin-dependent kinase 2 (CDK2) is induced by doxorubicin therapy, leading to cardiomyocyte cell death.[10,11] In the study published in this issue of *JACC: CardioOncology*, they explored the downstream effector signaling of CDK2 activation.[12] They hypothesized that retinoblastoma-like 2 peptide (RBL2/p130), an endogenous inhibitor of CDK2, would mitigate CDK2-driven cardiac injury from anthracyclines. Using genetic murine models of retinoblastoma 2 (rbl2) knockout and overexpression, they found that knockout of rbl2 increased CDK2 expression and enhanced anthracycline toxicity both in vivo and in studies of cultured cardiomyocytes in vitro. In vivo, cardiac contractile dysfunction was increased, heart weight was decreased, and myocardial fibrosis was increased following 1 month of weekly doxorubicin therapy (5 mg/kg/wk). In vitro, down-regulation of rbl2 led to CDK2-dependent forkhead box O1

*Editorials published in *JACC: CardioOncology* reflect the views of the authors and do not necessarily represent the views of *JACC: CardioOncology* or the American College of Cardiology.

From the [a]Division of Cardiology, Department of Internal Medicine, Pauley Heart Center, Virginia Commonwealth University, Richmond, Virginia, USA; [b]Section on Cardiovascular Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; [c]Section on Comparative Medicine, Department of Pathology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; [d]Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, Virginia, USA; [e]Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, Virginia, USA; and the [f]Medical Service of the McGuire Veterans Affairs Medical Center, Richmond, Virginia, USA.

Kathryn Ruddy, MD, served as the Guest Editor for this paper.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2666-0873 https://doi.org/10.1016/j.jaccao.2023.01.008

(FOXO1)-mediated increase in the mitochondria-localized proapoptotic protein bim, with evidence of increased cardiomyocyte programmed cell death and initial data to implicate dysfunctional mitochondria in the pathway. Anthracycline-induced CDK2-mediated activation of FOXO1 increased expression of *rbl2* mRNA with initial increases in rbl2 content (net protective) but followed by decreased FOXO1 mediated rbl2 protein stability (potential depletion of rbl2 leading to additional cardiomyocyte injury). Overall, the investigators conclude that the net FOXO1-driven increase in *rbl2* gene expression represents an attempt at protective adaptation, though the potential complexity is apparent and provides possible insight into their human studies of rbl2 polymorphisms. Appropriate models were used to establish the mechanistic pathway, with the minor limitations that the model of anthracycline toxicity was acute; molecular studies were performed in neonatal, not adult, cardiomyocytes; and the genetic manipulations were not cardiomyocyte specific. Also, the up- or down-regulation of rbl2 content was present from birth and thus present during development, rather than conditionally induced only during adulthood. However, the alteration of rbl2 during the entire life span would model the subsequent study of human rbl2 polymorphisms.

Nonetheless, the investigators also supply intriguing initial human data that alteration in rbl2 function likely affects clinical sensitivity to anthracycline cardiac toxicity. There are 6 missense variants in the human *RBL2* gene with allele frequencies >0.1%.[12] One, the rs17800727 G allele (A>G), is the most common, with a frequency >21%. This G allele has a lower expression of rbl2 in the human left ventricle and is surprisingly associated with a decreased risk for anthracycline cardiomyopathy, lowest in homozygotes for the G allele in contrast to a 3-fold increased risk for anthracycline cardiomyopathy in AA homozygotes.[12] The traction to human disease is important, though the complexity of the association between rbl2 and cardiomyopathy is complex, as initial interpretation would conclude that the human findings of reduced rbl2 content in the left ventricle seen with the G allele should exacerbate, not reduce, cardiac risk. The investigators speculate that, consistent with the murine results, the G allele might lead to lower CDK2-FOXO1 activity than the presence of the A allele. Although these results certainly require further validation given the relatively small number of patients in each of the 2 clinical cohorts, the results merit further genetic study given the potential to provide a prospective risk index for anthracycline-induced cardiac toxicity.

The study of Xia et al[12] is important in several aspects. First, CDK2 is identified as a mediator upstream of the mitochondria and topoisomerase 2β,[7,8] as well as oxidative mitochondrial metabolism mechanisms.[2,4] The possible regulation of topoisomerase 2β and mitochondrial electron transport by the CDK2-rbl2-FOXO1 axis should be considered. As the investigators speculate, the potential to modulate CDK2 in order to increase rbl2 (protective) expression in contrast to FOXO1 (deleterious) is a possible approach to cardiac protection[12] and certainly merits future investigation. Of equal importance, it becomes critical to evaluate whether potential known cardioprotective interventions against anthracycline cardiac toxicity, including remote preconditioning,[13] moderate exercise,[14] or pharmacologic therapy,[15,16] affect the CDK2 signaling axis.

The present study of CDK2 signaling via FOXO1 and bim yet again highlights the importance of the mitochondria and places emphasis on the mitochondria as a major effector of anthracycline toxicity. Thus, potential approaches to chronically modulate mitochondrial metabolism to mitigate oxidant- and calcium-driven myocyte injury via the use of hydrogen sulfide,[17,18] nitrite and nitrate production,[2] or even pharmacologic modulation of mitochondrial complex I[19,20] deserve future consideration. Also, the potential to protect nonmyocyte cells in the myocardium, including fibroblasts, needs to be evaluated, as anthracyclines directly phenoconvert fibroblasts into myofibroblasts and promote the secretion of excess collagen, promoting interstitial myocardial fibrosis, independent of cardiomyocyte death and replacement fibrosis.[21,22]

The CDK2-rbl2-FOXO1 pathway may provide a future opportunity to prospectively assess the potential for anthracycline-mediated cardiac toxicity,[10,11] allowing the selection of chemotherapeutic agents or doses tailored to individual patient risk. It will be especially important to assess if clinically accessible tissues, including white blood cells,[23] or perhaps even skeletal muscle,[24] can serve as tissue surrogates for the heart to assess these key signaling pathways to use biochemical and molecular techniques to assess the patient-specific risk for chemotherapy-induced cardiac toxicity.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by grant 1R21AG071249-01A1 (Drs Lesnefsky and Chen) from the National Institute on Aging, the U.S. Department

JACC: CARDIOONCOLOGY, VOL. 5, NO. 3, 2023
JUNE 2023:374–376

of Veterans Affairs Office of Research and Development Medical Research Service Merit Review Award (2IO1BX001355-09A2; Drs Chen and Lesnefsky), and National Heart, Lung and Blood Institute grant K01HL145329 (Dr Meléndez). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Edward J. Lesnefsky, Cardiology Section, Medical Service 111(J) McGuire VA Medical Center, 1201 Broad Rock Boulevard, Richmond, Virginia 23249, USA. E-mail: edward.lesnefsky@va.gov.

REFERENCES

1. Sawyer DB. Anthracyclines and heart failure. *N Engl J Med*. 2013;368:1154–1156.

2. Zhu SG, Kukreja RC, Das A, Chen Q, Lesnefsky EJ, Xi L. Dietary nitrate supplementation protects against Doxorubicin-induced cardiomyopathy by improving mitochondrial function. *J Am Coll Cardiol*. 2011;57:2181–2189.

3. Koleini N, Nickel BE, Edel AL, Fandrich RR, Ravandi A, Kardami E. Oxidized phospholipids in doxorubicin-induced cardiotoxicity. *Chem Biol Interact*. 2019;303:35–39.

4. Ichikawa Y, Ghanefar M, Bayeva M, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest*. 2014;124:617–630.

5. Fang X, Wang H, Han D, et al. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci U S A*. 2019;116:2672–2680.

6. Chang HC, Shapiro JS, Ardehali H. Getting to the "heart" of cardiac disease by decreasing mitochondrial iron. *Circ Res*. 2016;119:1164–1166.

7. Vejpongsa P, Yeh ET. Topoisomerase 2β: a promising molecular target for primary prevention of anthracycline-induced cardiotoxicity. *Clin Pharmacol Ther*. 2014;95:45–52.

8. Zhang S, Liu X, Bawa-Khalfe T, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. 2012;18:1639–1642.

9. Yeh ETH, Ewer MS, Moslehi J, et al. Mechanisms and clinical course of cardiovascular toxicity of cancer treatment I. Oncology. *Semin Oncol*. 2019;46:397–402.

10. Xia P, Liu Y, Chen J, Coates S, Liu DX, Cheng Z. Inhibition of cyclin-dependent kinase 2 protects against doxorubicin-induced cardiomyocyte apoptosis and cardiomyopathy. *J Biol Chem*. 2018;293:19672–19685.

11. Xia P, Chen J, Liu Y, Fletcher M, Jensen BC, Cheng Z. Doxorubicin induces cardiomyocyte apoptosis and atrophy through cyclin-dependent kinase 2-mediated activation of forkhead box O1. *J Biol Chem*. 2020;295:4265–4276.

12. Xia P, Chen J, Sapkota Y, et al. *RBL2* regulates cardiac sensitivity to anthracycline chemotherapy. *J Am Coll Cardiol CardioOnc*. 2023;5(3):360–373.

13. Gertz ZM, Cain C, Kraskauskas D, et al. Remote ischemic pre-conditioning attenuates adverse cardiac remodeling and mortality following doxorubicin administration in mice. *J Am Coll Cardiol CardioOnc*. 2019;1:221–234.

14. Kang DW, Wilson RL, Christopher CN, et al. Exercise cardio-oncology: exercise as a potential therapeutic modality in the management of anthracycline-induced cardiotoxicity. *Front Cardiovasc Med*. 2021;8:805735.

15. He D, Hu J, Li Y, Zeng X. Preventive use of beta-blockers for anthracycline-induced cardiotoxicity: a network meta-analysis. *Front Cardiovasc Med*. 2022;9:968534.

16. Lewinter C, Nielsen TH, Edfors LR, et al. A systematic review and meta-analysis of beta-blockers and renin-angiotensin system inhibitors for preventing left ventricular dysfunction due to anthracyclines or trastuzumab in patients with breast cancer. *Eur Heart J*. 2022;43:2562–2569.

17. Li Z, Xia H, Sharp TE III, et al. Mitochondrial H_2S regulates BCAA catabolism in heart failure. *Circ Res*. 2022;131:222–235.

18. Mauro AG, Mezzaroma E, Toldo S, et al. NLRP3-mediated inflammation in cardio-oncology: sterile yet harmful. *Transl Res*. 2023;252:9–20.

19. Mohsin AA, Chen Q, Quan N, et al. Mitochondrial complex I inhibition by metformin limits reperfusion injury. *J Pharmacol Exp Ther*. 2019;369:282–290.

20. He L, Jiang Y, Liu K, et al. Insights into the impact of a membrane-anchoring moiety on the biological activities of bivalent compounds as potential neuroprotectants for Alzheimer's disease. *J Med Chem*. 2018;61:777–790.

21. Meléndez GC, Hundley WG. Is myocardial fibrosis a new frontier for discovery in cardiotoxicity related to the administration of anthracyclines? *Circ Cardiovasc Imaging*. 2016;9:e005797.

22. Levick SP, Soto-Pantoja DR, Bi J, et al. Doxorubicin-induced myocardial fibrosis involves the neurokinin-1 receptor and direct effects on cardiac fibroblasts. *Heart Lung Circ*. 2019;28:1598–1605.

23. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Molina AJ. Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol*. 2016;10:65–77.

24. Gorgey AS, Graham ZA, Chen Q, et al. Sixteen weeks of testosterone with or without evoked resistance training on protein expression, fiber hypertrophy and mitochondrial health after spinal cord injury. *J Appl Physiol (1985)*. 2020;128:1487–1496.

KEY WORDS cardiotoxicity, cyclin-dependent kinase 2 (CDK2), doxorubicin, reactive oxygen species, retinoblastoma-like peptide 2 (RBL2), topoisomerase 2