Editorial

Visnagin—a new protectant against doxorubicin cardiotoxicity? Inhibition of mitochondrial malate dehydrogenase 2 (MDH2) and beyond

Lei Xi

Pauley Heart Center, Division of Cardiology, Virginia Commonwealth University, Richmond, VA 23298-0204, USA

Correspondence to: Lei Xi, MD, FAHA. Associate Professor, Division of Cardiology, Box 980204, Virginia Commonwealth University, 1101 East Marshall Street, Room 7-020C, Richmond, VA 23298-0204, USA. Email: lei.xi@vcuhealth.org.

Submitted Oct 08, 2015. Accepted for publication Oct 13, 2015.
doi: 10.3978/j.issn.2305-5839.2015.10.43
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.10.43

Doxorubicin (DOX) is a broad-spectrum and potent anthracycline antibiotic that has been widely used since 1960s as a chemotherapeutic agent to treat a variety of human cancers (1). Despite its superior anti-cancer efficacy, the clinical use of DOX is often limited by dose-dependent cardiotoxicity, which may lead to irreversible dilated cardiomyopathy and congestive heart failure (2,3). Currently predominant theories for explaining DOX cardiotoxicity include the DOX-induced increase of oxidative stress in cardiomyocytes (4), alteration of mitochondrial energetics (5,6), and direct effect on DNA. Particularly, anthracyclines promote the formation of reactive oxygen species (ROS) through redox cycling of their aglycones and anthracycline-iron complexes (7). Other suggested contributing factors to DOX cardiotoxicity comprise platelet-activating factor, prostaglandins, histamine, calcium, and C-13 hydroxy anthracycline metabolites, etc. (8,9). DOX is metabolized to doxorubicinol, which is implicated for its cardiotoxicity, possibly by causing perturbation of the iron homeostasis (4).

Mitochondria are abundant in cardiomyocytes and may take up to 35% of the cell volume. Since cardiac cells rely upon mitochondria-generated ATP to sustain dynamic contractile function, any interference with structural or functional integrity of mitochondria is likely to cause selective toxicity to the heart (5). Evidently, mitochondria have been considered as the primary targets for DOX cardiotoxicity (5,6). Accumulation of DOX aglycones in the inner membrane of mitochondria can interfere with electron carriers of the respiratory chain and cause the release of cytochrome c (10). DOX also disturb calcium homeostasis in cardiac mitochondria (11). DOX cardiotoxicity is associated with excessive ROS generation in mitochondria (12,13).

Due to the complex multi-factorial cellular and molecular drivers underlying DOX cardiotoxicity, the optimal therapeutic approaches for protection against DOX cardiotoxicity have not yet been identified, despite over 40 years of extensive research. Notably Herman *et al.* in 1972 first introduced bisdioxopiperazine compound as a cardioprotective agent against DOX cardiotoxicity (14). The subsequent research in this area led to identification of dexrazoxane, the only drug currently approved by the United States Food and Drug Administration (FDA) for reducing DOX cardiotoxicity (15). Dexrazoxane acts by displacing iron from anthracycline-iron complexes or by chelating free cellular iron and in turn preventing the site-specific iron-catalyzed ROS overproduction (7). However, critical reassessment of the so-called "ROS and iron" hypothesis indicated that numerous exogenous antioxidants failed to alleviate DOX cardiotoxicity in clinical trials (7). Several chelators that are stronger and more selective for iron than dexrazoxane did not protect against DOX cardiotoxicity (16,17). Another concern about dexrazoxane is its adverse effects of worsening myelosuppression and interfering with the anti-cancer efficacy of DOX, resulting in increased risk of secondary malignancy in pediatric patients with Hodgkin's lymphoma (18). Therefore, there is an ongoing and urgent need to search for a better and safer cardioprotectant against DOX toxicity.

In an article published on December 10, 2014 in *Science Translational Medicine*, entitled "Visnagin protects against doxorubicin-induced cardiomyopathy through modulation of mitochondrial malate dehydrogenase" (19), Liu and

colleagues from Massachusetts General Hospital and Harvard Medical School elegantly demonstrated protective effects of visnagin against DOX cardiotoxicity. These investigators first established a zebrafish model of DOX-induced cardiomyopathy (indicated by cardiac myocyte apoptosis and contractility decline) and screened ~3,000 compounds using this model. They found that visnagin and diphenylurea rescue the DOX-induced cardiac dysfunction and/or circulatory defects in zebrafish and mice. Importantly, visnagin did not reduce the chemotherapeutic efficacy of DOX in several cultured tumor cell lines, zebrafish, and mouse xenograft models (19).

First, in terms of methodological advances, Liu *et al*. (19) and a few other groups (20,21) have utilized zebrafish as an effective model for high-throughput screening a number of synthetic or natural chemical formula that possess protective effects against DOX cardiotoxicity. These simple and low-cost zebrafish models of tractable heart failure represent a good alternative approach differed from the much more expensive mammalian models for large scale and non-biased drug discovery process targeting the complex heart failure pathologies, e.g., DOX cardiomyopathy in adult zebrafish and aristolochic acid-caused cardiac defects in zebrafish embryos (19-21). It is plausible to predict that this kind of approaches may lead to additional discoveries of novel drug candidates for prevention and treatment of DOX cardiotoxicity.

One of the exciting findings from the work of Liu *et al*. is the identification of mitochondrial malate dehydrogenase (MDH2) as a novel target for reducing DOX cardiotoxicity (19). By means of affinity chromatography, these researchers revealed that visnagin binds to MDH2, a key enzyme in the tricarboxylic acid cycle. They further demonstrated that treatment with other known structurally diverse inhibitors of MDH2 (i.e., mebendazole, thyroxine, iodine, malate) also prevented DOX cardiotoxicity, suggesting that modulation of MDH2 activity is responsible for cardioprotective effects of visnagin. MDH are active enzymes in glyoxysomes, mitochondria, peroxisomes, chloroplasts and the cytosol (22) that interact with the malate-aspartate shuttle (MAS), which constitutes the primary metabolic pathway for transfer of reducing equivalents from the cytosol into the mitochondria for oxidation. The MAS enzymatic reactions include the coupled cytosolic and mitochondrial transamination of aspartate and glutamate and inter-conversion of malate and oxaloacetate by MDH. The enzymes of MAS co-localize with enzymes of the tricarboxylic acid cycle at the inner mitochondrial membrane. MAS also shares intermediates with the tricarboxylic acid cycle (oxaloacetate, malate, α-ketoglutarate) and the electron transport chain (glutamate, malate) ensuring a tight co-ordination between shuttle activity and mitochondrial respiration (23). The flux of MAS is tightly linked to the flux of the tricarboxylic acid cycle and the electron transport chain and MAS is also an important regulator of cytosolic and mitochondrial calcium homeostasis. It was reported that inhibition of MAS during ischemia and early reperfusion led to down-regulation of mitochondrial respiration during lethal ischemia and in turn afforded cardioprotective effects similar to ischemic preconditioning (23). Hence, it is conceivable that visnagin induces cardioprotection against DOX cardiotoxicity—a type of non-ischemic tissue injury, likewise via inhibition of MDH2 and in turn the MAS.

Remarkably, an inhibitor of MDH2 such as visnagin may also be beneficial against resistance to chemotherapy in cancer cells. A recent study showed that MDH2 confers docetaxel resistance in prostate cancer via regulations of JNK signaling and oxidative metabolism (24). It was shown that MDH2 is overexpressed in clinical prostate cancer specimens. Patients with MDH2 overexpression had a significantly shorter period of relapse-free survival after chemotherapy. Whereas MDH2 expression was elevated in prostate cancer cell lines compared to benign prostate epithelial cells, stable knockdown of MDH2 via shRNA in the cancer cells decreased cell proliferation and increased docetaxel sensitivity. More recently, Lo *et al*. reported that MDH2 plays a role in the development of DOX-resistant uterine cancer (25). Therefore, the potential benefits in both cardioprotective and anti-cancer fronts would make visnagin more desirable as an adjunct agent for cancer chemotherapy.

Nevertheless, it remains relatively uncertain if MDH2 inhibition is the only mechanism underlying the visnagin-induced cardioprotection. Previous studies from Duarte and colleagues demonstrated a significant vasodilatory effect of visnagin that can lead to a systemic hypotensive response to *in vivo* administration of this compound (26,27). Interestingly, our previous studies showed that several chemical or natural compounds with vasodilatory property can also afford protective effects against DOX cardiotoxicity in mice. One of the cardioprotective modalities is dietary supplementation of inorganic nitrate, which improves post-DOX ventricular contractile function, cell survival, and mitochondrial respiratory chain function (28,29). We also revealed cardioprotective effects of phosphodiesterase 5 (PDE5) inhibitors—sildenafil (30) and tadalafil (31), two well-known vasodilators, against DOX

cardiotoxicity. Intriguingly, visnagin is also reported to be a weak pan-inhibitor of PDE (32). The exact contribution of the vasoactive effects of visnagin during its *in vivo* administration to the drug-induced cardioprotection remains to be evaluated.

In addition, as a possible mechanistic perspective, it will be interesting to find out whether or not vasnagin and other MDH2 inhibitors affect topoisomerase 2 (Top2)—a key regulator of DNA replication, transcription, and recombination. Recent studies from Dr. Yeh's group at the MD Anderson Cancer Center in Houston, Texas demonstrated that DOX inhibits both Top2α (found in the rapidly proliferating tumor cells) and Top2β (found in the less actively dividing cells, such as cardiomyocytes) and inhibition of Top2β plays a key role in mediating DOX cardiotoxicity (33,34). These investigators also showed that Top2β deletion protected mice from cardiotoxic effects of DOX. Therefore, based on the demonstrated important role played by Top2β in development of DOX cardiotoxicity (35), Top2β should be considered as a potential target for cardioprotective therapy, including the use of visnagin.

After all, can we translate the cardioprotective effects of visnagin observed in zebrafish and rodents (19) into an effective cardioprotectant in helping cancer patients receiving DOX chemotherapy? The ultimate answer depends upon a series of rigorous pre-clinical validation in larger mammalian species as well as clinical trials in cancer patients for attesting the therapeutic usefulness of visnagin against DOX cardiotoxicity in humans. In terms of translational value, it is noteworthy that visnagin is a natural product extracted from the plant *Ammi Visnaga*, which grows wild in the Eastern Mediterranean countries. This compound was traditionally prescribed by Arabic folk physicians as a diuretic and antispasmodic in cases of ureteral stones and it was also studied in animal models for ameliorating angina pectoris (36). The potential utilities of such botanical derivatives as reported by Liu *et al*. on visnagin (19) and by our laboratory on inorganic nitrate (beetroot juice) (29,37-39) deserve further studies and validations, in order to develop them into potentially effective and affordable therapies for protecting thousands of cancer patients undergoing DOX chemotherapy against its devastating cardiotoxicity and eventually to improve the clinical outcome and quality of life of the cancer survivors.

Acknowledgements

None.

Footnote

Provenance: This is a Guest Editorial commissioned by Section Editor Ran Mo, MD (Department of Cardiothoracic Surgery, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China).
Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Bristow MR, Mason JW, Billingham ME, et al. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy, and cardiac catheterization. Ann Intern Med 1978;88:168-75.
2. Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Engl J Med 1998;339:900-5.
3. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer 2003;97:2869-79.
4. Minotti G, Menna P, Salvatorelli E, et al. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 2004;56:185-229.
5. Wallace KB. Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis. Cardiovasc Toxicol 2007;7:101-7.
6. Lebrecht D, Kokkori A, Ketelsen UP, et al. Tissue-specific mtDNA lesions and radical-associated mitochondrial dysfunction in human hearts exposed to doxorubicin. J Pathol 2005;207:436-44.
7. Simůnek T, Stérba M, Popelová O, et al. Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. Pharmacol Rep 2009;61:154-71.
8. Olson RD, Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. FASEB J 1990;4:3076-86.
9. Thorn CF, Oshiro C, Marsh S, et al. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet Genomics 2011;21:440-6.
10. Clementi ME, Giardina B, Di Stasio E, et al. Doxorubicin-derived metabolites induce release of cytochrome C and inhibition of respiration on cardiac isolated mitochondria. Anticancer Res 2003;23:2445-50.
11. Solem LE, Heller LJ, Wallace KB. Dose-dependent increase in sensitivity to calcium-induced mitochondrial dysfunction and cardiomyocyte cell injury by doxorubicin.

J Mol Cell Cardiol 1996;28:1023-32.
12. Berthiaume JM, Wallace KB. Adriamycin-induced oxidative mitochondrial cardiotoxicity. Cell Biol Toxicol 2007;23:15-25.
13. Chaiswing L, Cole MP, Ittarat W, et al. Manganese superoxide dismutase and inducible nitric oxide synthase modify early oxidative events in acute adriamycin-induced mitochondrial toxicity. Mol Cancer Ther 2005;4:1056-64.
14. Herman EH, Mhatre RM, Lee IP, et al. Prevention of the cardiotoxic effects of adriamycin and daunomycin in the isolated dog heart. Proc Soc Exp Biol Med 1972;140:234-9.
15. Hasinoff BB, Hellmann K, Herman EH, et al. Chemical, biological and clinical aspects of dexrazoxane and other bisdioxopiperazines. Curr Med Chem 1998;5:1-28.
16. Hasinoff BB, Patel D, Wu X. The oral iron chelator ICL670A (deferasirox) does not protect myocytes against doxorubicin. Free Radic Biol Med 2003;35:1469-79.
17. Hasinoff BB, Patel D. The iron chelator Dp44mT does not protect myocytes against doxorubicin. J Inorg Biochem 2009;103:1093-101.
18. Tebbi CK, London WB, Friedman D, et al. Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J Clin Oncol 2007;25:493-500.
19. Liu Y, Asnani A, Zou L, et al. Visnagin protects against doxorubicin-induced cardiomyopathy through modulation of mitochondrial malate dehydrogenase. Sci Transl Med 2014;6:266ra170.
20. Wang L, Zhang X, Chan JY, et al. A novel danshensu derivative prevents cardiac dysfunction and improves the chemotherapeutic efficacy of doxorubicin in breast cancer cells. J Cell Biochem 2016;117:94-105.
21. Huang CC, Monte A, Cook JM, et al. Zebrafish heart failure models for the evaluation of chemical probes and drugs. Assay Drug Dev Technol 2013;11:561-72.
22. Gietl C. Malate dehydrogenase isoenzymes: cellular locations and role in the flow of metabolites between the cytoplasm and cell organelles. Biochim Biophys Acta 1992;1100:217-34.
23. Nielsen TT, Støttrup NB, Løfgren B, et al. Metabolic fingerprint of ischaemic cardioprotection: importance of the malate-aspartate shuttle. Cardiovasc Res 2011;91:382-91.
24. Liu Q, Harvey CT, Geng H, et al. Malate dehydrogenase 2 confers docetaxel resistance via regulations of JNK signaling and oxidative metabolism. Prostate 2013;73:1028-37.
25. Lo YW, Lin ST, Chang SJ, et al. Mitochondrial proteomics with siRNA knockdown to reveal ACAT1 and MDH2 in the development of doxorubicin-resistant uterine cancer. J Cell Mol Med 2015;19:744-59.
26. Duarte J, Pérez-Vizcaíno F, Torres AI, et al. Vasodilator effects of visnagin in isolated rat vascular smooth muscle. Eur J Pharmacol 1995;286:115-22.
27. Duarte J, Torres AI, Zarzuelo A. Cardiovascular effects of visnagin on rats. Planta Med 2000;66:35-9.
28. Xi L, Zhu SG, Hobbs DC, et al. Identification of protein targets underlying dietary nitrate-induced protection against doxorubicin cardiotoxicity. J Cell Mol Med 2011;15:2512-24.
29. Zhu SG, Kukreja RC, Das A, et al. Dietary nitrate supplementation protects against Doxorubicin-induced cardiomyopathy by improving mitochondrial function. J Am Coll Cardiol 2011;57:2181-9.
30. Fisher PW, Salloum F, Das A, et al. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. Circulation 2005;111:1601-10.
31. Koka S, Das A, Zhu SG, et al. Long-acting phosphodiesterase-5 inhibitor tadalafil attenuates doxorubicin-induced cardiomyopathy without interfering with chemotherapeutic effect. J Pharmacol Exp Ther 2010;334:1023-30.
32. Duarte J, Lugnier C, Torres AI, et al. Effects of visnagin on cyclic nucleotide phosphodiesterases and their role in its inhibitory effects on vascular smooth muscle contraction. Gen Pharmacol 1999;32:71-4.
33. Zhang S, Liu X, Bawa-Khalfe T, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med 2012;18:1639-42.
34. Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. J Am Coll Cardiol 2014;64:938-45.
35. Vejpongsa P, Yeh ET. Topoisomerase 2β: a promising molecular target for primary prevention of anthracycline-induced cardiotoxicity. Clin Pharmacol Ther 2014;95:45-52.
36. Anrep GV, Barsoum GS, Kenawy MR, et al. Ammi visnaga in the treatment of the anginal syndrome. Br Heart J 1946;8:171-7.
37. Daiber A, Gori T, Münzel T. Inorganic nitrate therapy improves Doxorubicin-induced cardiomyopathy a new window for an affordable cardiovascular therapy for everyone? J Am Coll Cardiol 2011;57:2190-3.
38. Das A, Durrant DE, Kukreja RC, et al. Oral ingestion of beetroot juice reduces doxorubicin cardiotoxicity

via enhancement of nitric oxide, protein kinase G and Aldehyde Dehydrogenase 2. Circulation 2012;126:A11130.
39. Yin C, Rehman S, Kukreja RC, et al. Upregulation of cardiac microRNA 34a and Sirt1, Notch1 mRNA/protein expression following oral ingestion of beetroot juice: cardioprotective role against doxorubicin toxicity. Circulation 2014;130:A12237.

Cite this article as: Xi L. Visnagin—a new protectant against doxorubicin cardiotoxicity? Inhibition of mitochondrial malate dehydrogenase 2 (MDH2) and beyond. Ann Transl Med 2016;4(4):65. doi: 10.3978/j.issn.2305-5839.2015.10.43

