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Twisting and ironing: Doxorubicin cardiotoxicity by mitochondrial DNA damage

Karin C. Nitiss^{1,2} and John L. Nitiss^{1,2,*}

¹ Biomedical Sciences Department, UIC College of Medicine, Rockford

² Department of Biopharmaceutical Sciences, UIC College of Pharmacy

Summary

Anthracyclines are active clinical agents that have multiple mechanisms of cytotoxicity. Cardiotoxicity by anthracyclines limits the therapeutic potential of these agents, but mechanisms leading to cardiotoxicity remain controversial. Transgenic mice that lack mitochondrial topoisomerase I are hypersensitive to doxorubicin cardiotoxicity, providing support for cardiotoxicity arising from damage of mitochondrial DNA.

In this issue of Clinical Cancer Research, Pommier and colleagues have added an important new piece to the puzzle of understanding how anthracyclines contribute to cardiotoxicity. They found that mice lacking mitochondrial topoisomerase I (Top1mt) were much more sensitive to doxorubicin cardiotoxicity than littermates that expressed this enzyme (1). A very important conclusion of this work is that mitochondrial DNA damage likely plays a major role in doxorubicin-mediated cardiotoxicity. This conclusion provides a framework for reconciling many of the contradictory models for how this critical side effect of anthracyclines arises, and provides new approaches for prospective prediction of anthracycline cardiotoxicity.

Anthracyclines such as doxorubicin have been mainstays in cancer chemotherapy, with notable successes in the treatment of both solid malignancies and leukemias. Since the discovery of the anti-cancer properties of anthracyclines, there have been nearly continuous controversies concerning how anthracyclines kill cancer cells. The two important hypotheses that have guided our current thinking on anthracycline cytotoxicity are (1) anthracyclines act by targeting topoisomerases (the “Twist” in the title of this commentary) and (2) Fe mediated generation of reactive oxygen species. The recognition that anthracyclines had a major side effect of cardiotoxicity has also led to a bewilderingly complex number of suggestions concerning the mechanisms of cardiotoxicity (see (2-5) for divergent perspectives on how anthracyclines cause cardiotoxicity).

* Correspondence to: John L. Nitiss UIC College of Pharmacy at Rockford 1601 Parkview Ave. Rockford, IL 61107 jlnitiss@uic.edu 815-395-5583.

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Authors' Contributions

The conception, writing, and revision of this manuscript were jointly carried out by Karin C. Nitiss and John L. Nitiss.

Doxorubicin metabolism has been extensively studied, and doxorubicin and its metabolites generate a variety of cellular effects leading to generation of reactive oxygen species (ROS), changes in iron metabolism, and changes in Ca^{2+} signaling (**Figure 1**). ROS generation through redox cycling, and the importance of iron in these processes has been areas of major emphasis. While studies with antioxidants as cardioprotective agents have generally been disappointing, the hypothesis that chelation of iron could reduce cardiotoxicity has led to the acceptance of dexrazoxane as an important clinically useful cardioprotective agent (6). Dexrazoxane is an iron chelator that is structurally similar to EDTA; however, dexrazoxane has additional effects as described below that obscure a simple conclusion that iron chelation alone is responsible for cardioprotection.

In addition to iron chelation, dexrazoxane is a catalytic inhibitor of DNA topoisomerase II (Top2). Doxorubicin is a potent inhibitor of Top2, and causes the trapping of the enzyme on DNA as a covalent complex. Since a substantial body of evidence suggests that topoisomerase mediated damage is a critical determinant of tumor cell killing (7), could topoisomerase II be related to doxorubicin cardiotoxicity as well? Liu and colleagues showed that treatment of non-dividing cells with dexrazoxane leads to depletion of Top2 β , the only Top2 isoform that is normally expressed in non-dividing cells (and therefore the only Top2 isoform in cardiomyocytes). Therefore, dexrazoxane leads to the elimination of Top2 β , and prevents trapping of the enzyme on DNA (reviewed in (7)). These observations were explored further in a mouse model where Top2 β was selectively depleted in cardiomyocytes. Cardiomyocytes lacking Top2 β did not exhibit transcriptional patterns associated with doxorubicin mediated damage, and importantly, showed reduced levels of mitochondrial damage, and reduced cardiomyopathy (8).

To reconcile these hypotheses, it is helpful to note that one major area of consensus is that both short term and chronic cardiotoxicity of doxorubicin is most clearly seen in effects on mitochondria. Therefore, an appealing hypothesis is that doxorubicin induces cardiotoxicity through effects on mitochondrial DNA.

Mitochondrial DNA encodes a small number of proteins that are critical for oxidative phosphorylation, and the RNAs that make up the mitochondrial ribosome. All other components of the mitochondrial “genome” are encoded in the nucleus. Proteins destined for the mitochondrion that are encoded by the nucleus including a mitochondrial DNA polymerase metabolism, transcription proteins, ribosomal proteins, as well as some proteins critical for oxidative phosphorylation. DNA metabolic proteins encoded by the nucleus include a type 1B topoisomerase that appears to be specific for mitochondria (9), and two other topoisomerases, Top3 α (a type 1A topoisomerase that has functions distinct from other topoisomerases) and Top2 β (10).

With these considerations in mind, we can reconsider the effects of doxorubicin specifically on mitochondrial DNA. By redox and iron dependent mechanisms, ROS will generate lesions in mitochondrial DNA. Oxidative lesions will block mitochondrial transcription and replication and is likely also to impact retrograde signaling from the mitochondrion to the nucleus. Alternately, since mitochondria contain Top2 β , doxorubicin will lead to trapped Top2 complexes, and ultimately single and double strand breaks in DNA. Mitochondrial

DNA as the focus of doxorubicin cytotoxicity also provides a clear explanation of the protective action of dexrazoxane. The ability of dexrazoxane to chelate iron reduces the generation of free radicals, reducing ROS dependent DNA damage. While it is unclear whether the proteasome dependent degradation of Top2 β induced by dexrazoxane will effectively eliminate this enzyme from mitochondria, overall enzyme levels likely will be reduced, thereby preventing topoisomerase-mediated DNA damage.

How does the mitochondrial Top1 provide protection from these two sources of DNA damage to mitochondria? As noted in the paper by Pommier in this issue (1), deletion of mitochondrial Top1 does not lead to an elevation of Top2 β . If this were the case, then the higher expression of Top2 β would lead to higher levels of mitochondrial DNA damage. However, even though neither Top2 β nor mitochondrial Top1 are essential for mitochondrial DNA replication, transcription, or genome maintenance, elimination of one of these enzymes increases the reliance on the remaining enzyme. As previously found in yeast, deletion of nuclear Top1 makes cells hypersensitive to doxorubicin and etoposide even though the expression level of Top2 does not change (7). Therefore, one likely mechanism for the enhanced cardiotoxicity in cells lacking mitochondrial Top1 is the reliance on an enzyme that is sensitive to doxorubicin. In this regard, it would also be interesting to determine whether mice lacking mitochondrial Top1 are more sensitive to dexrazoxane. It is possible that the degradation of Top2 β induced by dexrazoxane will lead to severe mitochondrial DNA damage due to an almost complete lack of topoisomerase activity.

Mechanistic studies with doxorubicin always have complicated subplots, and the observations reported by Pommier and colleagues also carry a potential objection. While mitochondrial topoisomerase I is localized specifically to mitochondria, Pommier and co-workers previously observed that deletion of mitochondrial Top1 leads to higher levels of ROS, accompanied by elevated levels of **nuclear** γ H2AX foci (11). While the paper in the current issue indicates that deletion of top1mt leads to lower levels of doxorubicin induced ROS, the higher constitutive level of ROS previously reported (in MEFs, not in cardiomyocytes (11)) slightly complicates the picture. Importantly, Pommier and colleagues did not see changes in ROS levels in this work, which examined cardiomyocytes. Nonetheless, a consistent hypothesis relating ROS to cardiotoxicity could argue that loss of top1mt leads to an elevation in ROS, further exacerbated by doxorubicin. The reason why this model disappoints is that it provides no satisfying explanation of the observation that Top2 β is required for cytotoxicity.

We would like to be able to say that cardiotoxicity by doxorubicin and other anthracyclines is caused by mechanism “x”, but the complications of this active anti-cancer drug likely keeps us away from such simple conclusions. Instead, the work reported in this issue by Pommier and co-workers likely directs us in a more productive direction. Since a gene such as top1mt has a profound effect on doxorubicin cardiotoxicity, pharmacogenomics approaches that enable us to predict patients that require special care when being treated with anthracyclines will enable us to extend the overall benefits and minimize the risks of patients treated with these agents. These insights should also be expanded to other recently predicted genes that affect anthracycline cytotoxicity such as a recent observation that mice deficient in the iron storage protein ferritin are also more sensitive to doxorubicin than wild

type mice (12). The application of these insights will likely lead us to the conclusion that a consideration of multiple mechanisms will be needed to fully appreciate how doxorubicin results in cardiotoxicity.

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Figure.

Doxorubicin and other anthracyclines cause a wide range of cellular damage. Previous work had demonstrated the importance of targeting nuclear DNA, both by the generation of reactive oxygen species, and by inhibition of topoisomerase II (Top2). The work by Pommier and colleagues in this issue highlights the importance of the ability of anthracyclines to generate damage to mitochondrial DNA, especially in cardiomyocytes. Their observation that mitochondrial topoisomerase I (Top1mt) is important for tolerating the DNA damage in mitochondria supports the hypothesis that mitochondrial DNA damage is highly relevant to cardiomyopathy, and suggests that this enzyme and perhaps other enzymes important for mitochondrial DNA metabolism may be useful as pharmacogenomic markers for the likelihood of anthracycline-induced cardiomyopathy.