HHS Public Access

Author manuscript

*Biochemistry*. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

*Biochemistry*. 2018 April 24; 57(16): 2294–2296. doi:10.1021/acs.biochem.8b00337.

# An Emerging Pathway of Doxorubicin Cardiotoxicity Mediated through CYP2J2

**William R. Arnold**[1] and **Aditi Das**[*,†,1,2,3,4]

[†]Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana IL 61801

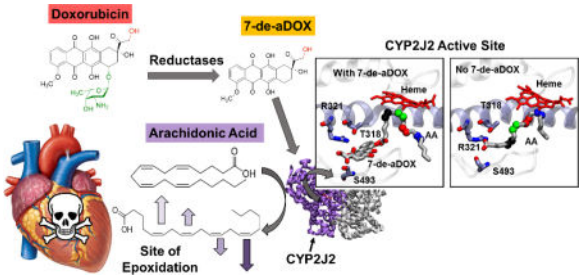
[1]Department of Biochemistry, University of Illinois Urbana-Champaign, Urbana IL 61801

[2]Center for Biophysics and Computational Biology, University of Illinois Urbana-Champaign, Urbana IL 61801

[3]Beckman Institute for Advanced Science and Technology, University of Illinois Urbana-Champaign, Urbana IL 61801

[4]Department of Bioengineering, Neuroscience program, University of Illinois Urbana-Champaign, Urbana IL 61801

## Graphical abstract




Doxorubicin (DOX) is among the oldest and most-used chemotherapeutics. However, its use is limited by a cumulative dose that leads to cardiotoxicity. DOX is an anthracycline quinone that is known to produce reactive oxygen species (ROS). Although ROS play a function in mediating the cardiotoxicity, there remain elusive alternative mechanisms that govern its specific cardiotoxicity. This is exemplified by the fact that 5-iminodaunorubicin (5-IDN) and zorubicin (ZRN) are relatively non-cardiotoxic analogues, even though ZRN forms ROS as efficiently as DOX and 5-IDN produces much fewer ROS.[1] Having a better understanding of the mechanisms underlying DOX cardiotoxicity will be beneficial in the development of efficacious and nontoxic analogues of DOX for cancer chemotherapy.

Cytochrome P450 (CYP) 2J2 is the most highly expressed CYP of the myocardium. It is responsible for the biosynthesis of anti-inflammatory and vasodilatory epoxide metabolites from endogenous fatty substrates. CYP2J2 is well-known for converting arachidonic acid

[*]Corresponding Author: To whom correspondence should be addressed: Aditi Das, Ph.D., University of Illinois Urbana-Champaign, 3836 VMBSB, 2001 South Lincoln Avenue, Urbana IL 61802, Phone: 217-244-0630. aditidas@illinois.edu.

(AA), an ω-6 polyunsaturated fatty acid (PUFA), into four regioisomers of epoxyeicosatrienoic acids (EETs) (Figure 1). These EETs reduce myocardial infarct size, reduce ischemia-reperfusion injury, and prevent arrhythmia.[2] For these reasons, they are cardioprotective. CYP2J2 is also responsible for the metabolism of several drugs, many of which are cardiotoxic. DOX cardiotoxicity was previously shown to be reduced by either the overexpression of CYP2J2 or the administration of EETs.[3]

We therefore hypothesized that DOX directly modulates CYP2J2-mediated AA metabolism, which contributes to cardiotoxicity.[4] In order to show this, we used an LC-MS/MS method to quantify the four regioisomers of EETs produced by CYP2J2 and its redox partner cytochrome P450 reductase (CPR). We found that DOX potently inhibits AA metabolism by CYP2J2 (Figure 1). We determined that the inhibition of AA metabolism by DOX is best described as competitive, thereby demonstrating that AA and DOX compete for binding to the active site of CYP2J2. DOX is known to be reduced by the obligate redox partner of CYP2J2, CPR to produce 7-deoxydoxorubicin aglycone (7-de-aDOX) (Figure 1). We therefore also investigated the effects of 7-de-aDOX on AA metabolism. We found that 7-de-aDOX partially inhibits AA metabolism, reducing activity to only 50% even at saturating concentrations (Figure 1). In addition to inhibiting AA metabolism, DOX and 7-de-aDOX have a concentration-dependent effect on the regioselectivity of EETs produced, increasing especially the amount of 5,6-EET and 8,9-EET (Figure 1). These EETs have downstream effects that differ from 11,12-EET and 14,15-EET.[2] E.g, 5,6-EET was shown to not be effective at promoting the survival of human lung microvascular endothelial cells and 8,9-EET was shown to be the least cardioprotective.[2, 5] This is the first study demonstrating that a ligand binding directly at the active site of CYP2J2 alters the site of AA metabolism.[4]

As a comparison, we also investigated the effects of the non-cardiotoxic analogues 5-IDN and ZRN on AA metabolism. We found that 5-IDN and ZRN demonstrate competitive inhibition of AA metabolism. They, however, do not have a significant effect on the site of metabolism like DOX or 7-de-aDOX have.

As a further comparison, we also investigated the effects of DOX and 7-de-aDOX on the metabolism of ebastine (EBS) by CYP2J2. Due to the inherent difficultly of measuring EETs, studies investigating CYP2J2 activity are often performed using a probe substrate such as EBS. Interestingly, we found that whereas DOX competitively inhibits EBS metabolism, 7-de-aDOX has no effect on the binding or metabolism of EBS. These data demonstrate that the effects on the regioselectivity of CYP2J2 by DOX/7-de-aDOX are unique to AA as a substrate.

To probe the molecular basis for these experimental observations, we performed molecular dynamics (MD) simulations with DOX/7-de-aDOX and AA in the active site of CYP2J2. We had previously used MD simulations to discover a key pocket in the CYP2J2 active site that mediates PUFA binding to CYP2J2. We found that 7-de-aDOX is capable of binding into this PUFA binding pocket, which concurrently alters the binding of AA (Figure 1). The altered positioning of AA in the CYP2J2 active site correlates to the changes in the site of metabolism we measure experimentally. DOX, however, does not bind this pocket and instead occupies most of the active site and prevents the binding of AA. This corroborates

the competitive inhibition we observe experimentally. Finally, 7-de-aDOX and EBS do not have overlapping binding sites, explaining the observation that 7-de-aDOX has no effect on the binding and metabolism of EBS.

In conclusion, we propose the following model of how DOX modulates AA metabolism through CYP2J2. DOX competitively inhibits AA metabolism by CYP2J2. This would reduce the amount of EETs available to combat the cardiotoxic assault initiated by DOX. DOX is then transformed by CPR into 7-de-aDOX. This metabolite binds CYP2J2 in the PUFA binding pocket to alter the binding conformation of AA. The result is a change in the site of metabolism to promote the production of EETs that are less effective at combating cardiotoxicity, building houses of straw rather than houses of bricks against the cardiotoxicity. This change in the homeostasis could further exacerbate the toxicity. Non-cardiotoxic analogues of DOX inhibit CYP2J2 but do not significantly alter the regioselectivity. This combined approach of *in vitro* kinetic experimentation and *in silico* analysis provides insights into a potentially new pathway of DOX toxicity, namely through the inhibition of EETs and modulation of the site of AA metabolism. These data form the foundation by which further *in vivo* investigations may study the cardiotoxicity of DOX. Furthermore, these data can aid the design of other analogues that have reduced cardiotoxicity, which do not modulate CYP2J2's AA metabolism.

## Acknowledgments

Funding Sources:

American Heart Association Scientist Development grant [15SDG25760064] and NIH R01 GM1155884

## References

1. Singh Y, Ulrich L, Katz D, Bowen P, Krishna G. Structural requirements for anthracycline-induced cardiotoxicity and antitumor effects. Toxicol Appl Pharmacol. 1989; 100:9–23. [PubMed: 2763305]
2. Xu X, Zhang XA, Wang DW. The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. Adv Drug Deliv Rev. 2011; 63:597–609. [PubMed: 21477627]
3. Zhang Y, El-Sikhry H, Chaudhary KR, Batchu SN, Shayeganpour A, Jukar TO, Bradbury JA, Graves JP, DeGraff LM, Myers P, Rouse DC, Foley J, Nyska A, Zeldin DC, Seubert JM. Overexpression of CYP2J2 provides protection against doxorubicin-induced cardiotoxicity. Am J Physiol Heart Circ Physiol. 2009; 297:H37–46. [PubMed: 19429816]
4. Arnold WR, Baylon JL, Tajkhorshid E, Das A. Arachidonic Acid Metabolism by Human Cardiovascular CYP2J2 is Modulated by Doxorubicin. Biochemistry. 2017; 56(51):6700–6712. [PubMed: 29200270]
5. Gross GJ, Hsu A, Falck JR, Nithipatikom K. Mechanisms by which epoxyeicosatrienoic acids (EETs) elicit cardioprotection in rat hearts. J Mol Cell Cardiol. 2007; 42:687–691. [PubMed: 17217955]

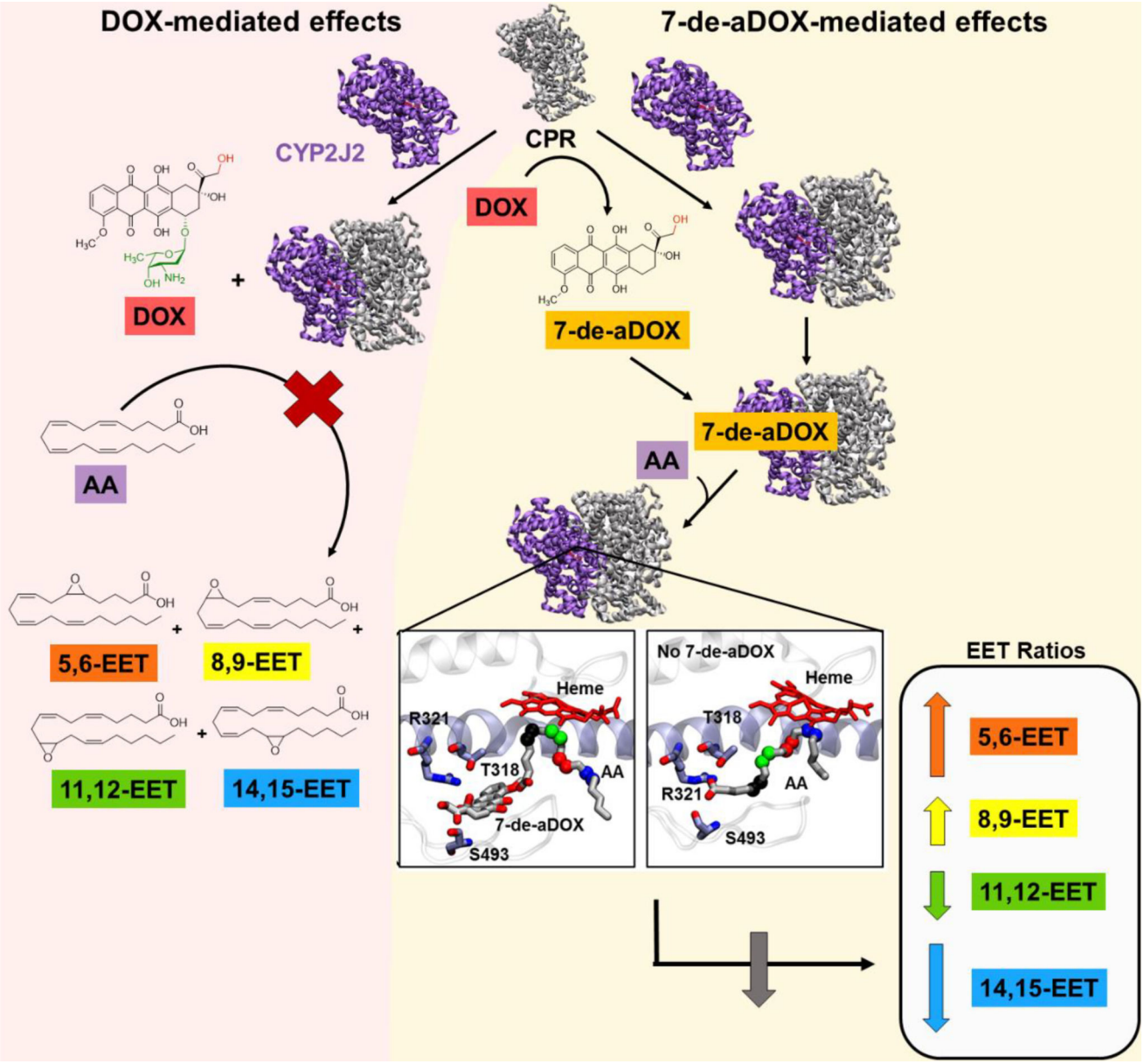



**Figure 1.**

Summary of the effects of doxorubicin (DOX)and 7-deoxydoxorubicin aglycone (7-de-aDOX) on AA metabolism by CYP2J2. Experimental and molecular dynamics simulations reveal the following. *Left panel.* DOX competitively inhibits arachidonic acid (AA) metabolism into epoxyeicosatrienoic acids (EETs) by CYP2J2. *Right panel* CPR metabolizes DOX to 7-de-aDOX. 7-de-aDOX binds into the binding sitealong with AA. The binding of 7-de-aDOX alters the positioning of AA, which changes the preferred site of metabolism increasing the ratio of 5,6-EET produced and decreasing the amount of 14,15-EET.