This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Adriamycin and Its "Heart-breaking" Free Radical Formation

by

Liang Zhang

Department of Anatomy and Cell Biology
The University of Iowa
Iowa City, IA 52242-1109

For 77:222, Spring 2005

14. March 2005

Abbreviations

ADR:	Adriamycin
$ADR^{\bullet -}$:	Adriamycin semiquinone-free radical
EPR:	Electron paramagnetic resonance
Fe(II):	Ferrous iron
Fe(III):	Ferric iron
Flp:	Flavoprotein
ROS:	Reactive oxygen species

Outline

Abstract

Adriamycin (ADR) is an effective anticancer xenobiotic. However, the life-threatening cardiotoxicity of ADR greatly limits its clinical use. This cardiotoxicity is related to its property to be turned into a free radical inside cells. The free radical form of ADR generates harmful reactive oxygen species either by directly reacting with oxygen or through the mediation of iron. Therefore, understanding and preventing the free radical reactions of ADR are essential for developing strategies to ameliorate its side effects. This review will introduce the basic chemistry, as well as the detection of ADR and its free radical reactions.

Introduction

Adriamycin (ADR), also called doxorubicin, is a glycoside xenobiotic isolated from *Streptomyces peucetius* [1]. Since the first clinical trials in the late 1960s, ADR and its derivatives have been well accepted as a family of chemotherapeutic agents (anthracyclines) in the treatment of different carcinomas, sarcomas, and lymphomas. The mechanisms underlying the anticancer effect of ADR include DNA intercalation and interference with the function of DNA topoisomerase II [1]. Unfortunately, despite the anticancer effects, ADR induces both acute and chronic cardiotoxicity, which greatly limits its clinical use [2]. The exact mechanism behind this cardiotoxicity is still unclear, but it has been agreed that free radicals play an important role [3, 4]. Once inside the cells, ADR can be reduced to the adriamycin semiquinone free radical ($ADR^{\bullet -}$), which can directly damage nuclear DNA or generate reactive oxygen species (ROS) through the reaction with oxygen. In addition, the interaction between ADR and iron is also thought to be a free radicals producing pathway that will damage cellular lipids, DNA, and proteins [5]. A better understanding of the free radical reactions of ADR has become necessary for the development of anthracycline drug with less cardiotoxicity. In the following sections, I will discuss the basic chemistry, biochemistry, and detection methods for adriamycin, as well as its free radical reactions.

Chemistry of adriamycin

The structure of adriamycin (**Figure 1a**) consists of a water-soluble tetracyclic quinoid aglycone adriamycinone (14-hydroxydaunomycinone) linked to an aminosugar daunosamine through a glycosidic linkage [1]. The quinone structure inside the ADR molecule has the

potential to receive an electron, leading to the formation of $ADR^{\bullet -}$, a semiquinone-free radical **(Figure 1b)** [3]:

$$ADR + e^- \rightarrow ADR^{\bullet -} \quad (1)$$

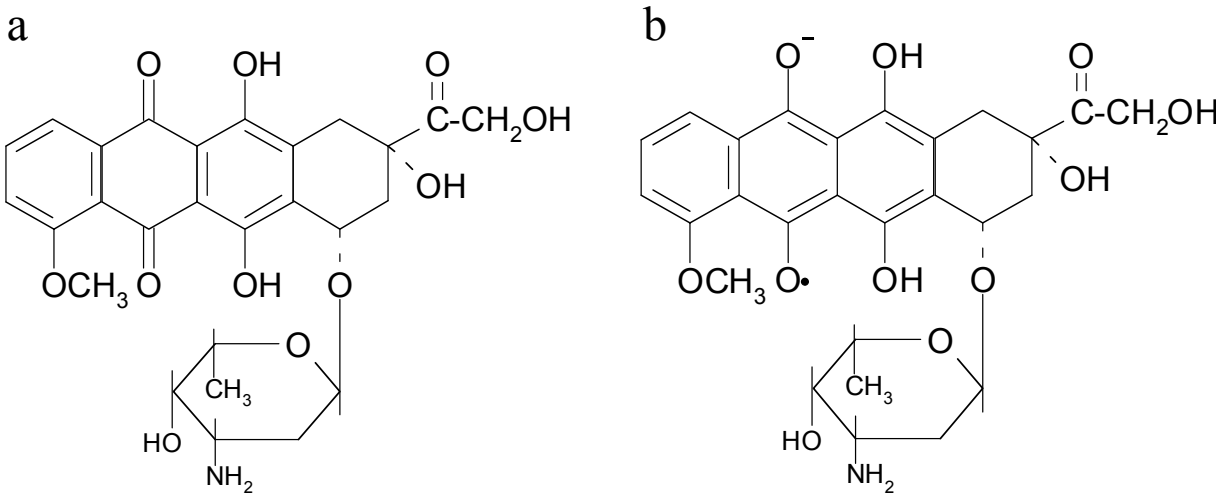

Figure 1. Structure of adriamycin (panel a) and adriamycin free radical (panel b). One-electron addition to the quinone structure in adriamycin leads to the formation of the semiquinone-free radical.

The standard one-electron reduction potential (E°′) for the ADR/ $ADR^{\bullet -}$ couple is -341 mV, at 25°C, pH 7.0 [1]. This value is more negative than the E°′ of the O_2/ $O_2^{\bullet -}$ couple, -160 mV. Therefore, based on their positions on the pecking order, one can predict the occurrence of the following reaction ($\Delta E°' = +181$ mV):

$$ADR^{\bullet -} + O_2 \rightarrow ADR + O_2^{\bullet -} \quad (2)$$

This reaction occurs with a large rate constant ($k = 10^8$ M^{-1} s^{-1}) [6], and forms the basis for the cardiotoxicity of ADR, which is discussed in more details below.

Another essential property of adriamycin is that it has the ability to bind to ferric ion (Fe(III)) in ADR-iron ratios of 1:1, 2:1, and 3:1. The formation constants for the association of

the first, second, and third ADR with Fe^{3+} have been determined to be 10^{18}, 10^{11}, and $10^{4.4}$ M^{-1}, respectively [7]. The atoms on adriamycin that participate in this chelating process are proposed to be one carbonyl and one phenolate oxygen **(Figure 2)**. The formation of ADR-iron complex *in vivo* is proposed to play an important role in the free radical reactions and cardiotoxicity of ADR [5].

Figure 2. Adriamycin forms a complex with iron. The carbonyl and phenolate oxygen are the atoms on adriamycin that are involved in this interaction. Derived from [5].

Biochemistry of adriamycin

After cellular uptake, ADR is primarily concentrated inside the nucleus. The anticancer effect of ADR is due to its high-affinity DNA intercalation and interference with the catalytic cycle of DNA topoisomerase II. This has been reviewed in detail [1, 8], while the focus of this review is on the cellular redox reactions of ADR that are related to its cardiotoxic effects.

As we mentioned above, the quinone structure in the ADR molecule makes it an electron acceptor of cellular flavoproteins (Flp), which are primarily localized in the microsomes and nucleus. Flavoproteins, such as NADPH cytochrome P-450 reductase, accept electrons from NADPH and donate them to ADR, leading to the formation of ADR semiquinone free radical [9]. In the presence of oxygen, the ADR radical donates its unpaired electron to oxygen, resulting in the formation of superoxide and regeneration of ADR. Thus ADR acts as an electron carrier between Flp and molecular oxygen for the production of superoxide. Bachur *et al.* have summarized this process in a schematic (**Figure 3**). This process is a potential mechanism for the harmful effects of ADR, because the presence of a small amount of ADR in an oxidative environment will consume NADPH and generate a lot of superoxide, leading to DNA damage mediated by ROS [10].

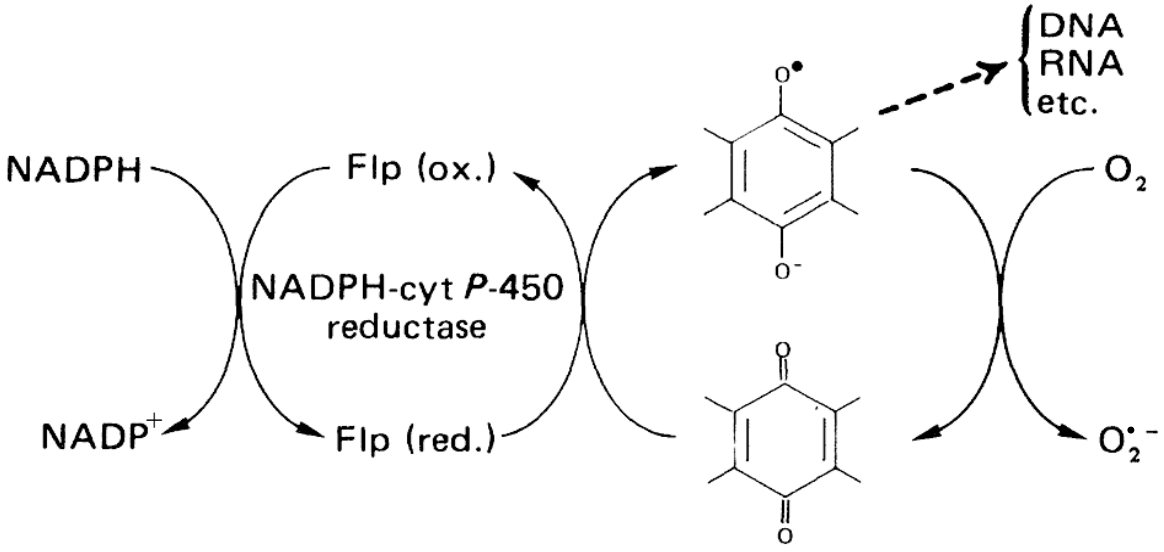

Figure 3. A scheme for adriamycin-mediated cellular superoxide generation. Adriamycin is converted to its free radical form after receiving an electron from flavoproteins, such as NADPH cytochrome P-450 reductase. The adriamycin free radical can transfer the electron to oxygen and make superoxide, which later leads to the formation of other ROS. In another mode, the adriamycin free radical can directly bind to biomolecules such as DNA and lead to damage through its interaction with iron. Modified from [10].

In addition to the above "redox-cycling" mechanism of free-form ADR, other evidence has suggested that the ADR-iron complex plays a significant role in the generation of free radicals and cellular toxicity of ADR [3, 5]. As mentioned above, ADR is able to form a strong complex with Fe(III). In cells, ADR can even extract Fe(III) from ferritin, which is the major storage of cellular iron and prevents the involvement of iron in harmful redox reactions [5]. Once the complex is formed, ADR free radical can reduce bound Fe(III) to Fe(II):

$$ADR^{\bullet -} + Fe(III) \rightarrow ADR + Fe(II) \quad (3)$$

The resulting ferrous ion can then participate in radical generation reactions, such as the Fenton reaction to produce the highly oxidizing hydroxyl radical:

$$Fe(II) + H_2O_2 \rightarrow HO^{\bullet} + HO^- + Fe(III) \quad (4)$$

Thus, the net reaction of (3) and (4) is:

$$ADR^{\bullet -} + H_2O_2 \xrightarrow{Fe(III)} ADR + HO^{\bullet} + HO^- \quad (5)$$

So Fe(III) acts as a catalyst in this process, allowing $ADR^{\bullet -}$ to react with hydrogen peroxide and generate highly active hydroxyl radical [3].

Detection of adriamycin

Adriamycin can be detected by its characteristic absorption in visible electronic spectral range. The spectrum of adriamycin varies depending on the pH of the solution (**Figure 4**) [11]. In an aqueous solution with pH 2-7, adriamycin has $\lambda_{max} = 477$ nm and $\varepsilon_{477} = 9.3 \times 10^3\ M^{-1}\ cm^{-1}$. However, in basic solution with pH > 12, its λ_{max} shifts to 618 nm and $\varepsilon_{477} = 1.04 \times 10^4\ M^{-1}\ cm^{-1}$. The spectra-based method has been extensively utilized to detect its interaction with biomolecules such as DNA [11].

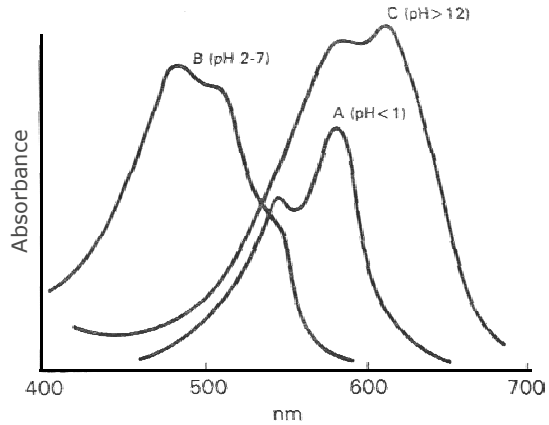

Figure 4. The visible absorption spectra of adriamycin. The features of the spectra of adriamycin vary depending on the pH of the solution. Modified from [11] and the authors did not show the values of the absorbance.

The electron paramagnetic resonance (EPR) technique has been applied by Kalyanaraman *et al.* to detect the generation of semiquinone-free radical of adriamycin [12]. In their study, xanthine oxidase was used to perform the one-electron reduction of adriamycin, and a specific one-line EPR spectrum was observed, where ΔHpp = 3.25 G, g = 2.0035 **(Figure 5).** This typical spectrum makes EPR an ideal approach to study the free radical reactions of adriamycin.

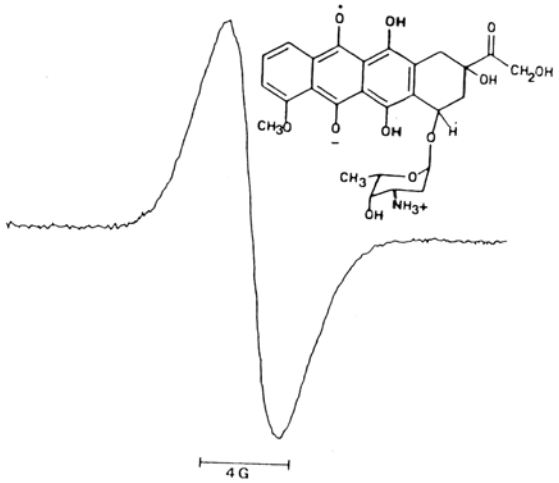

Figure 5. The EPR spectrum of the adriamycin free radical. The free radical was generated in a mixture of xanthine (400 μM), xanthine oxidase (0.2 units) and adriamycin (100 μM) in 2 mL phosphate buffer (100 mM, pH 7.5). The solution was puffed with nitrogen gas to avoid the production of superoxide. Conditions of spectrometer: modulation amplitude, 1 G; scan range, 40 G; and microwave power, 1 mW. From [12].

Summary and Conclusions

Adriamycin is an effective anticancer chemical yet with significant cardiotoxicity, which limits its clinical use. The cardiotoxicity is at least partially linked to the ability of adriamycin to generate free radicals. In a cell, adriamycin can directly act in a “redox-cycling” pattern to generate superoxide from oxygen or react with hydrogen peroxide to produce hydroxyl radical through the catalyzing by Fe(III), which it tightly binds to. Understanding the free radical properties of adriamycin will help us to develop approaches to promote its use in clinical cancer treatments.

References

1. Powis G. (1987) Anthracycline metabolism and free radical formation. In: Powis G, ed. *Metabolism and Action of Anti-cancer Drugs.* London: Taylor & Francis; pp 211-259.

2. Singal PK, Iliskovic N. (1998) Doxorubicin-induced cardiomyopathy. *N Engl J Med.* **339:**900-905.

3. Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. (1990) Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol Ther.* **47:**219-231.

4. DeGraff W, Hahn SM, Mitchell JB, Krishna MC. (1994) Free radical modes of cytotoxicity of adriamycin and streptonigrin. *Biochem Pharmacol.* **48:**1427-1435.

5. Minotti G, Cairo G, Monti, E. (1999) Role of iron in anthracycline cardiotoxicity: new tunes for an old song? *FASEB J.* **13:**199-212.

6. Kalyanaraman B, Perez-Reyes E, Mason RP. (1980) Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anticancer drugs. *Biochim Biophys Acta.* **630:**119-130.

7. May PM, Williams GK, Williams DR. (1980) Solution chemistry studies of adriamycin-iron complexes present *in vivo*. *Eur J Cancer.* **16:**1275-1276.

8. Young RC, Ozols RF, Myers CE. (1981) The anthracycline antineoplastic drugs. *N Engl J Med.* **305:**139-153.

9. Bachur NR, Gordon SL, Gee MV, Kon, H. (1979) NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc Natl Acad Sci U S A.* **76:**954-957.

10. Berlin V, Haseltine WA. (1981) Reduction of adriamycin to a semiquinone-free radical by NADPH cytochrome P-450 reductase produces DNA cleavage in a reaction mediated by molecular oxygen. *J Biol Chem* . **256:**4747-4756.

11. Gianni L, Corden BJ, Myers CE. (1983) The biochemical basis of anthracyclin toxicity and antitumor activity. In: Hodgson E, ed. *Reviews in Biochemical Toxicology.* New York: Elsevier Biomedical; pp 1-82.

12. Kalyanaraman B, Morehouse KM, Mason RP. (1991) An electron paramagnetic resonance study of the interactions between the adriamycin-semiquinone hydrogen peroxide, iron chelation, and radical scavengers. *Arch Biochem Biophys.* **286:**164-170.