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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

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ADRIAMYCIN

By

Haris Hamsakutty

B 180 ML

Free Radical and Radiation Biology
The University of Iowa
Iowa City, IA 52242-1181.

Tel: 319-335-8019

Fax: 319-335-8039

Email: haris-hamsakutty@uiowa.edu

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Abbreviations:

ADR: Adriamycin.
$ADR^{\bullet-}$: Adriamycin semiquinone free radical.
DNA: Deoxyribo nucleic acid.
EPR: Electron paramagnetic resonance.
Fe^{2+}: Fe(II), ferrous iron.
Fe^{3+}: Fe(III), ferric iron.
FP_{ox}: Oxidised flavoprotein.
FP_{red}: Reduced flavoprotein.
NADPH: Reduced nicotinamide adenine dinucleotide phosphate.

Abstract

Adriamycin, often known as doxorubicin, is an anthracycline antibiotic with cytotoxic actions. This cytotoxic action is used clinically in the treatment of cancer. Its clinical usefulness extends from hematological malignancies such as the acute leukemias and lymphomas to wide range of solid tumors including breast, lung and thyroid. It is capable of generating a variety of free radical species and this is considered to be important for its cytotoxic actions.The clinical use of this drug is limited by a dose dependant cardiac toxicity. This cardiotoxicity has been linked to free radical oxidative stress on the cardiac muscle. This review examines the role of this drug as a cytotoxic agent, with emphases on its free radical chemistry and biology.

Introduction.

Adriamycin is isolated from cultures of *Streptomyces peucetius var. caesius* [1]. It is characterized by a tetracyclic structure, a tetrahydronaphthacenequinone chromophore, linked to one or more sugar residues [2]. The sugar residue is very important for the anti tumour actions of adriamycin. The complete removal of this sugar moiety makes it ineffective as an anti tumor agent [2]. Several mechanisms have been proposed to explain for the antitumor properties of adriamycin. These include free radical mediated cytotoxicity through redox cycling of the ADR semiquinone radical, intercalation of DNA, stabilization of topoisomerase II- DNAcomplex and

also by inhibiting RNA and protein synthesis [3]. The ability of anthracyclines to undergo reduction to the semiquinone, followed by the redox cycling in the presence of O_2, forming $O_2^{\bullet-}$ and H_2O_2 is of significant importance in the antitumor actions.

Structure of Adriamycin.

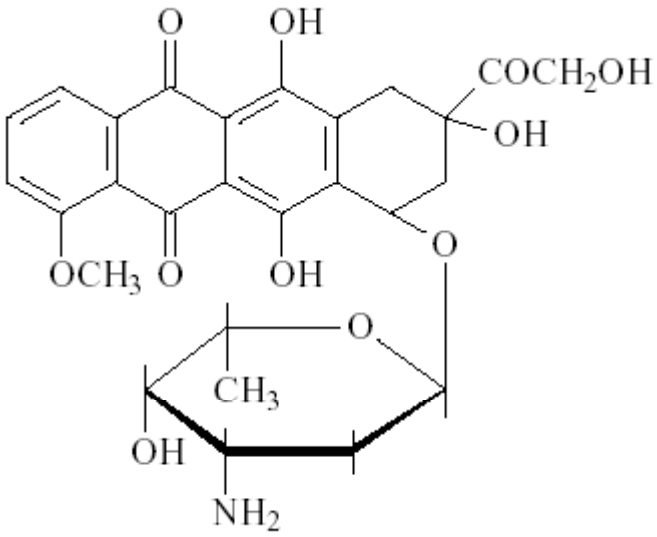

Figure 1. shows the structure of adriamycin. Adriamycin consists of a water soluble tetracycline quinoid aglycone, adriamycinone linked to the water soluble reducing aminosugar daunosamine [2].

Adriamycin is a glycoside that consists of the tetracyclic quinoid aglycone adriamycinone (14-hydroxydaunomycinone) linked to the amino sugar daunosamine [2]. It is isolated from cultures of *Streptomyces peucetius* as hydrochloride. The empirical formula for adriamycin hydrochloride is $C_{27}H_{29}O_{11}N$. HCl and the molecular weight is 579.88 g /mol. The pH of a 0.5% aqueous solution is 5.0 and the p*K*a determined by titration of adriamycin hydrochloride with 0.05 N sodium hydroxide is 8.22.

Solubility and stability.

Adriamycin is soluble in water, 0.9% saline and methanol. It is stable in the solid state and it can be stored for years at room temperature without any chemical changes or loss of activity [4].

Spectroscopic properties

Adriamycin has characteristic ultraviolet, visible and fluorescent spectra due to the nature of chromophore portion in its structure. The ultraviolet and visible absorption spectra of adriamycin in methanol have several maxima identified at 233, 253, 290, 477, 495, and 530 nm as shown in Figure 2 [5].

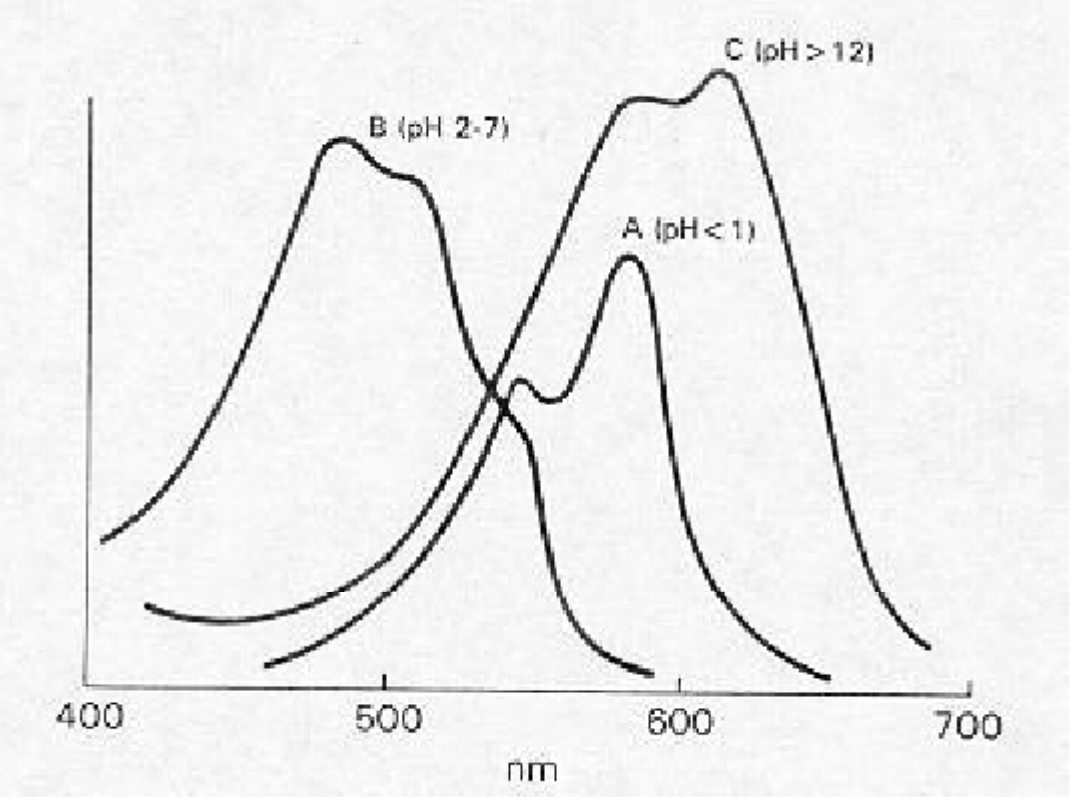

Figure 2. Visible absorption spectra of adriamycin in varying solutions [5]. Spectra A is adriamycin in concentrated acid and shows an extinction coefficient $\varepsilon_{582} = 3.7 \times 10^4\ M^{-1}\ cm^{-1}$ while spectra B shows, adriamycin in an aqueous solution with $\varepsilon_{477} = 9.3 \times 10^3\ M^{-1}\ cm^{-1}$. Spectra C is adriamycin in highly basic solution with $\varepsilon_{618} = 1.04 \times 10^4\ M^{-1}\ cm^{-1}$ [5].

The maxima and peak positions are solvent dependant. Adriamycin hydrochloride shows indicator like properties, turning from orange red to a blue violet color at pH 9, because of the

shift of absorption maxim to longer wave lengths in alkaline medium. This changes in visible absorption spectra is used to study the interactions of ADR with DNA.

EPR studies

Adriamycin and its metabolic derivatives can be studied using electron paramagnetic resonance (EPR). Adriamycin semiquinone is generated by the one electron reduction of ADR by xanthine oxidase. Figure 3 presents the spectrum of semiquinone free radical ($ADR^{\bullet -}$).

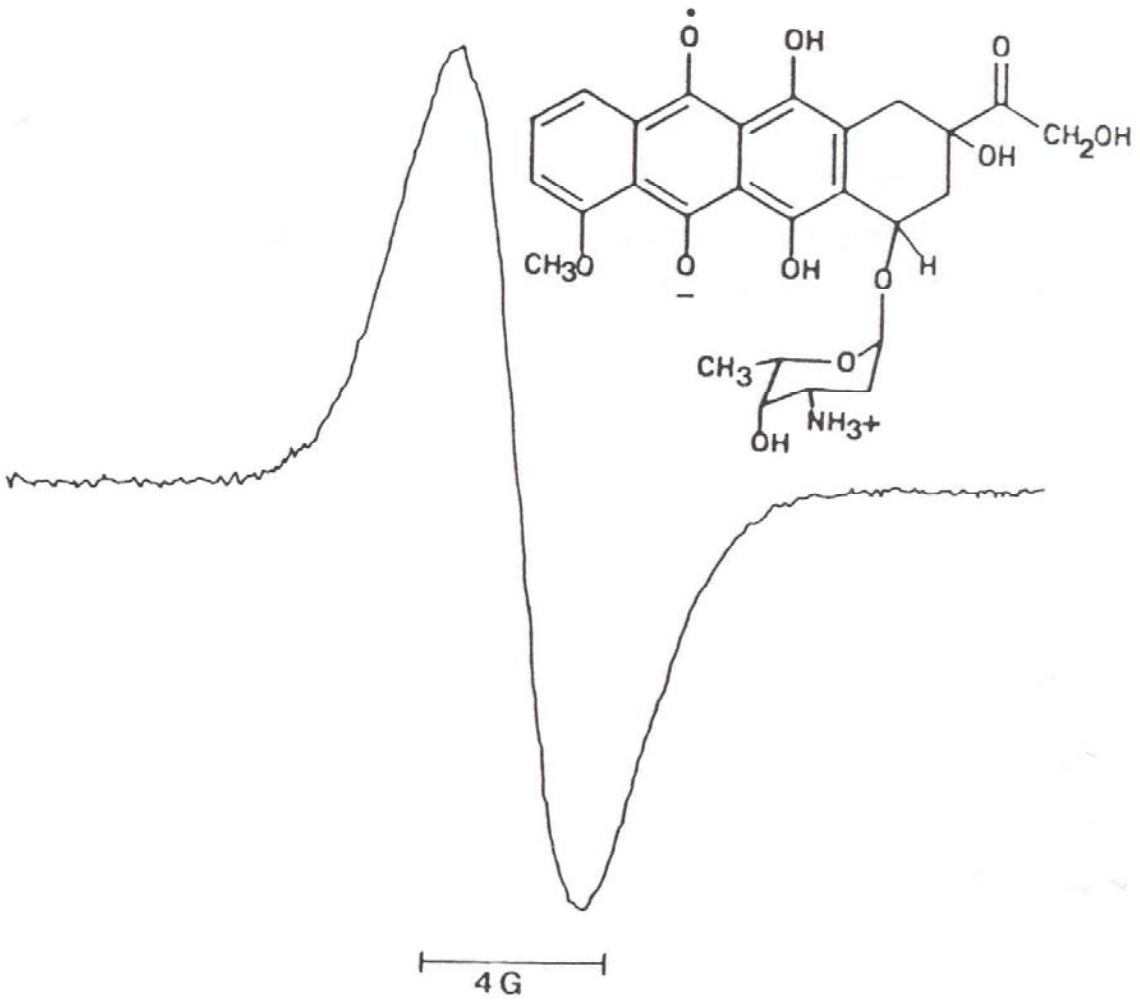

Figure 3: EPR spectrum and structure of Adriamycin semiquinone. A typical incubation mixture for EPR consisted of Adriamycin (100μM), xanthine (400μM), and xanthine oxidase (0.2 units)

in a 2 ml phosphate buffer (100 mM, pH 7.5). Spectrometer conditions: modulation amplitude1.0 G; microwave power, 1 mW; scan range, 40 G. (G: Gauss) [6].

Free radical reactions of adriamycin.

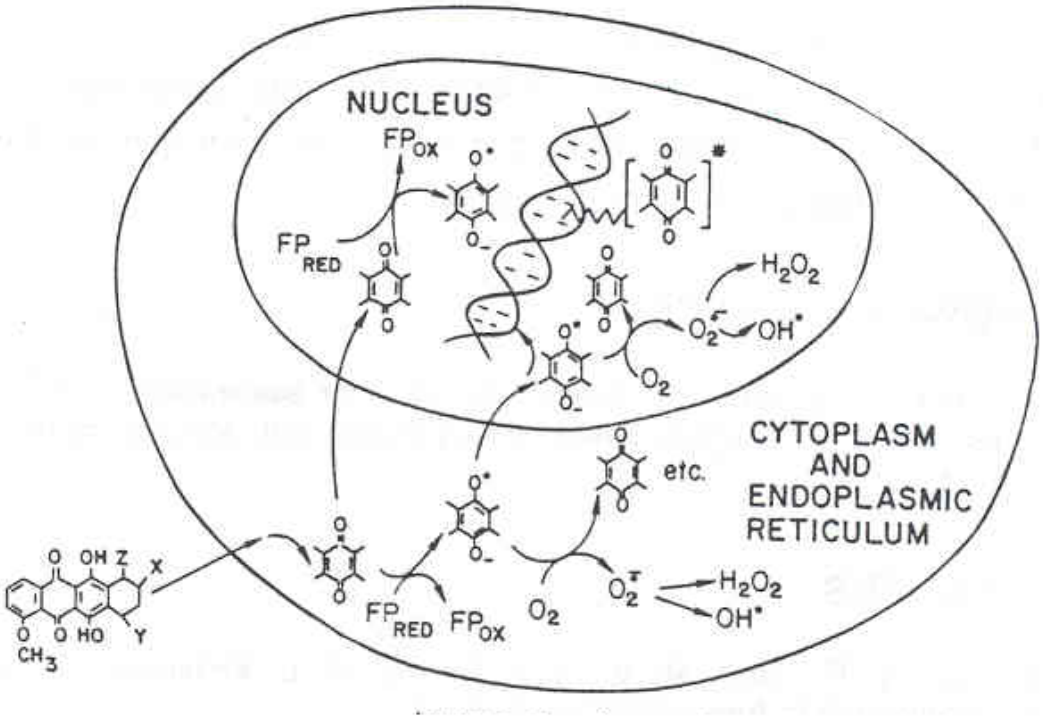

Figure 4. Shows the pathway of adriamycin into the cell. FP_{red} is the reduced flavoprotein and FP_{ox} is the oxidized flavoprotein. [7].

Studies by Bachur *et al* has proposed that ADR semiquinone radicals can be formed inside the nuclei by one electron reduction of ADR [7]. They postulated that ADR can enter the nucleus and be reduced by the flavoproteins located in the nuclear stroma (See figure 4). Enzymatic (flavin reductase) reduction of the anthracycline ring produces a semiquinone free radical ($ADR^{\bullet-}$) that can undergo redox cycle with O_2 to generate $O_2^{\bullet-}$ ($k = 10^8\ M^{-1}s^{-1}$). SOD will dismute $O_2^{\bullet-}$ to H_2O_2. This cascade of reactions will produce superoxide, hydrogen peroxide,

and hydroxide radical. The $ADR^{\bullet -}$ formed can directly damage the DNA or react with oxygen to produce reactive oxygen species that can damage the DNA and other nuclear materials [7].

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

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

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H \rightarrow H_2O_2 + O_2 \quad (3)$$

The ADR-iron complex can generate free radicals by two distinct mechanisms, one dependant on the presence of a reducing system and another independent of the reducing system. The enzymatic reduction occurs by NADH cytochrome P-450 reductase or by thiols like glutathione (GSH). In the absence of reducing system, ADR can directly reduce Fe(III) to Fe(II) in a radical driven Fenton reaction as shown in the reactions:

$$ADR^{\bullet -} + FE\ (III) \rightarrow Adr + Fe\ (II) \quad (4)$$

$$H_2O_2 + Fe\ (II) \rightarrow HO^{\bullet} + HO^- + Fe\ (III). \quad (5)$$

The free radicals generated results in DNA damage, lipid peroxidation, and protein inactivation and causes cytotoxicity.

Summary:

Adriamycin is a widely used anthracycline with very profound chemotherapeutics applications. Unfortunately, its clinical use is limited by cardiotoxicity. Adriamycin is capable of generating free radicals *in vivo*. This is considered to be crucial for the ani-tumor actions of adriamycin. Free radical independent mechanisms are also involved in the anti-tumor effects of adriamycin.

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