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Adriamycin: As Good as it Gets.

by

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Abbreviations:
ADR, adriamycin
ESR, electron spin resonance
Fe^{2+}, ferrous iron
Fe^{3+}, ferric iron
FP_{ox}, oxidized flavoprotein
FP_{red}, reduced flavoprotein
XO, xanthine oxidase

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Abstract:

Cancer is a devastating disease, however, there are many drugs that have been discovered that are very effective in treating cancer. Adriamycin, which is also known as Doxorubicin, is one such drug. It is a very effective chemotherapeutic with one very harmful side effect. Adriamycin, although effective in controlling most solid tumors, must be given in limited doses. This dose-limiting response is due to the cardiotoxicity of the drug *via* free radical interactions with the heart muscle cells. This drug has been and probably will continue being used until a drug is discovered or created that is similarly effective with less toxic side effects. This paper will focus on the structure, spectroscopic qualities and some free radical generating reactions of Adriamycin.

Introduction:

Cancer is a devastating disease to not only those people who are diagnosed but also to the families and friends of those people afflicted with the disease. Although there are many new ideas and treatments being studied every day, chemotherapy and radiation are still the most widely used treatments for the majority of cancers. Chemotherapy is the treatment that will be discussed in this paper. There are many types of chemotherapy drugs that are used depending on which type of cancer is diagnosed. Chemotherapeutic drugs are designed to interfere with the proliferation of cells by blocking the synthesis of DNA, RNA and/or proteins [3]. Doxorubicin is one of these chemotherapeutic drugs. Doxorubicin is also known as Adriamycin and will be referred to as such throughout the remainder of this paper. Adriamycin is used to treat many forms of cancer such as breast cancers, ovarian cancers, bone cancers, stomach cancers, lymphomas and multiple myelomas [8]. The number of patients that have been treated with Adriamycin is in the thousands or more since this drug has been used in chemotherapy for at least thirty years.

Structure and Background of Adriamycin:

Adriamycin is a member of the anthracyclin drug family. These drugs are quinone containing anti-tumor antibiotics [3]. Anthracyclin was first studied as a pigmented antibiotic, which had been discovered when produced by different strains of *streptomyces*. This discovery was made in the late 1950's [6]. The use of these anthracyclins as anti-tumor drugs started simultaneously in France, Italy and the USSR in 1963 [6]. In 1969 Adriamycin was isolated from a chemically mutated strain of *S. peucetius* [6]. It was shown that compared to the other anthracyclins, adriamycin had the greatest activity against solid tumors. Adriamycin, like other

chemotherapeutics acts in the tumor cell by binding to DNA and interfering with DNA replication and gene transcription [3]. This DNA interference causes strand breaks leading to the ultimate death of the cell. As with any chemotherapy drug some normal tissues are also affected. These affects result because of the block in synthesis in normal cells that proliferate quickly. Such blocks cause unwanted death of hematopoietic cells, intestinal cells and hair follicle cells. Adriamycin has other side affects besides the ones that come with most all chemotherapy drugs. These side affects include cardiomyopathy and loss of cardiac function. Although Adriamycin is a very effective anti-tumor drug its usefulness is limited by the dose-dependent cardiomyopathy that develops with its use [5]. This cardiac injury that occurs with use of the drug is probably because of the increased peroxidation of the lipids in the membrane [5]. There are many ways that scientists have of explaining the anti-tumor properties of adriamycin: intercalation of DNA, interaction with the membranes, bioreductive activation which leads to formation of drug and oxygen free radicals and the adriamycin-induced formation of $^{\bullet}OH$ [7]. This paper will focus on the free radical reactions and the induced formation of $^{\bullet}OH$. The structure of adriamycin is shown in Figure 1.

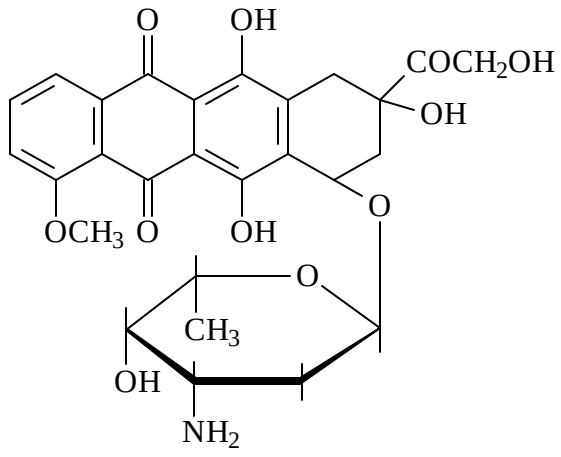

Figure 1: Structure of Adriamycin (Doxorubicin). Adapted from [3].

A one-electron reduction of adriamycin leads to the adriamycin semi-quinone free radical. This semi-quinone structure is shown in Figure 4. This free radical can be taken directly into the nucleus where it can be reduced by flavoproteins and react with the DNA in the cell to cause damage. This damage is good in the case of cancer cells but bad in the instance of damage to normal healthy cells. The scheme of how the radical semi-quinone may enter the cell is shown in Figure 2.

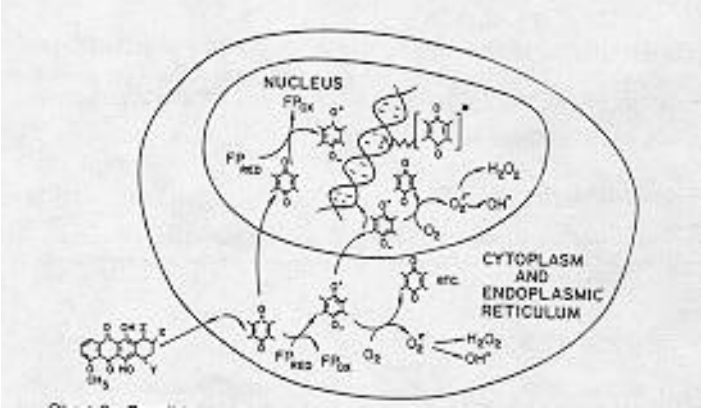

Figure 2: Possible reaction pathway of the quinone type drugs such as adriamycin into the cell. FP_{red} is the reduced flavoprotein and FP_{ox} is the oxidized flavoprotein. As can be seen the drug is taken into the cell and then shuttled to the nucleus via reductions from FP_{red}. This is how adriamycin binds with the DNA so well. Adapted from [1].

One of the side affects of using adriamycin is the cardiotoxicity. This toxicity of the drug is associated with the reduction to the semi-quinone [5]. The theory that this semi-quinone causes toxicity is reasonable because *in vivo* experiments have demonstrated that the severity of cardiotoxicity can be decreased by the use of free radical scavengers and iron chelators [5]. These types of reactions will be discussed more in the free radical reactions section of the paper.

Spectroscopy of Adriamycin:

The many forms of spectroscopy come in handy to help determine the structure and functions of adriamycin. The ultraviolet and visible absorption spectra of adriamycin in methanol have several maxima located at 233, 253, 290, 477, 495 and 530 nm; Figure 3 [4].

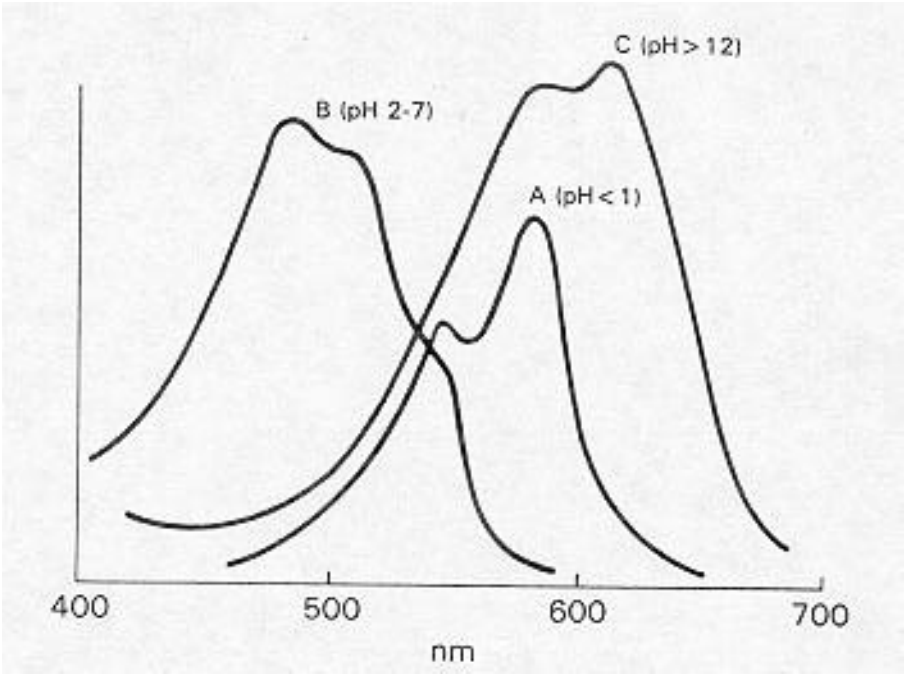

Figure 3: Visible absorption spectra of Adriamycin in varying solutions [4]. Spectra A is Adriamycin in concentrated acid and shows and extinction coefficient $\varepsilon_{582} = 3.7 \times 10^4\ M^{-1}\ cm^{-1}$, while spectra B shows Adriamycin in an aqueous solution with $\varepsilon_{477} = 9.3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

Fluorescence emission gives a spectrum in the range of 520-620 nm when excited at 485 and 253 nm [4]. The infrared spectrum shows multiple peaks at 3448, 2923, 1727, 1618, 1577 and 1445 cm^{-1} [4]. These different peaks correspond to the different quinone and ketone carbonyls of the adriamycin. Adriamycin semi-quinone is generated by adding xanthine oxidase to a nitrogen-purged solution containing xanthine and Adriamycin [5]. This reaction gives the ESR spectrum of the adriamycin semi-quinone, Figure 4.

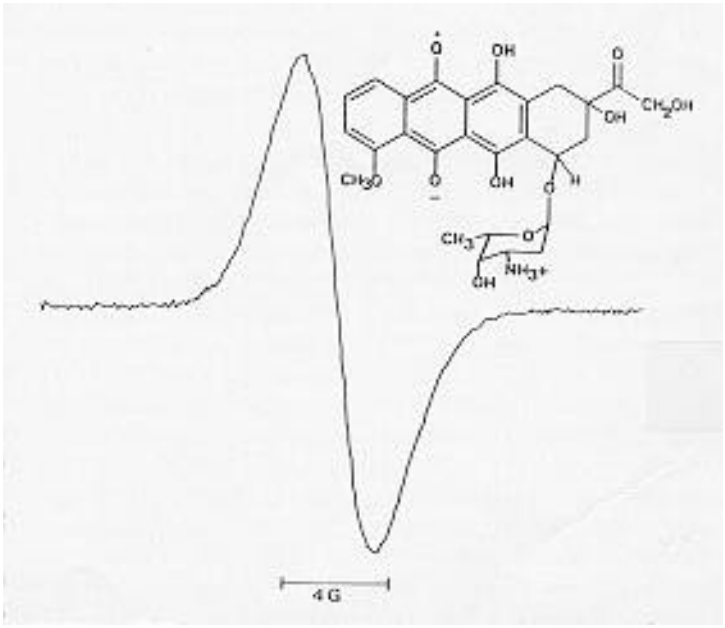

Figure 4: ESR spectrum and structure of the adriamycin semi-quinone. Adapted from [5]. The semi-quinone was generated by adding XO to a nitrogen-purged solution containing xanthine and adriamycin. This one line ESR spectrum occurred after a short lag time that corresponded to the removal of oxygen.

Free Radical Reactions of Adriamycin:

The acceptance of one electron causes the Adriamycin to be reduced to the Adriamycin free radical semi-quinone [2]. This semi-quinone free radical can by itself induce DNA damage [2]. The Adriamycin semi-quinone also will redox cycle with O_2 to produce $O_2^{\bullet-}$, $k = 10^8\ M^{-1}\ s^{-1}$ [5]. SOD will then dismute the $O_2^{\bullet-}$ to H_2O_2. The Adriamycin semi-quinone can also react to reduce ferric iron complexes in a radical-driven Fenton reaction [5]. Adriamycin semi-quinone reacts with H_2O_2 to produce $^{\bullet}OH$ in the presence of chelators. Without the presence of these chelators another oxidant that is yet unknown is formed [5]. These reactions of adriamycin semi-quinone are shown in Figure 5.

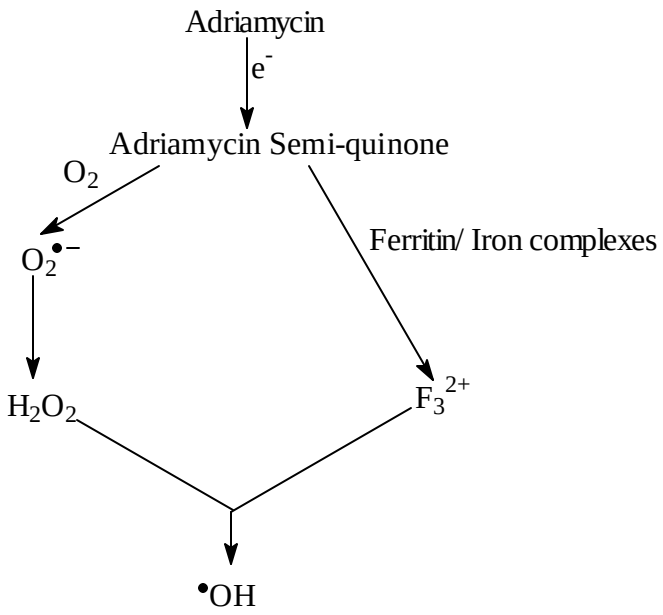

Figure 5: Adriamycin semi-quinone driven radical reactions. Adapted from [5].

However, in the presence of desferrioxamine, Adriamycin does not disappear when H_2O_2 is present [5]. This suggests that iron is important to the Adriamycin semi-quinone catalyzed reduction of H_2O_2 [5]. Adriamycin and Fe^{3+} react as shown in Figure 5 with an iron-binding constant of 10^{18} [3]. This implies that the adriamycin and iron are very tightly bound to each other. As seen above this is a problem because of the creation of hydroxyl radical through the reaction of adriamycin with iron and oxygen. Both of these substances are found in the body and are easily accessible to the adriamycin. These reactions are what lead to the toxic effect on cardiac cells in cancer treatment.

Summary:

Adriamycin is a very effective drug in treating solid tumor type cancers. This however is not enough to out weigh the detrimentally toxic effects to the heart that this drug causes *via* free radical interactions with oxygen and iron. The fact that iron is present in our bodies as well as

oxygen is what causes the problems. The reactions with these two substances both produce the hydroxyl radical, which is the most reactive radical. This radical attacks whatever it is near and is probably the major cause of the toxic effect to the heart. Adriamycin in small manageable doses however, is very beneficial to patients and should continue to be used in the treatment of cancers. The best possible scenario would be the ability to use adriamycin to effectively treat cancer cells while being able to protect the cardiac cells against the toxic effects of the radical reactions.

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