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Photogeneration of superoxide by adriamycin and daunomycin

An electron spin resonance and spin trapping study

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The hydroxyl and superoxide anion spin adducts of DMPO and 4-MePyBN, respectively, were obtained during photoirradiation of adriamycin and daunomycin solutions with visible light. Ethanol and dimethyl sulfoxide did not scavenge hydroxyl radicals in the photoirradiated drug solutions. Furthermore, the hydroxyl-DMPO spin adduct is not formed in the photolysis of air-free drug solutions, indicating that hydroxyl radicals are not directly produced in the photochemical reactions. Instead, the observed hydroxyl-DMPO is formed from the decay of the superoxide anion-DMPO spin adduct. The mechanism for generating the superoxide anion radical appears to be a direct electron transfer from the photoexcited adriamycin and daunomycin to dissolved oxygen.

Adriamycin *Daunomycin* *Superoxide* *Spin trapping* *DMPO* *4-MePyBN*

1. INTRODUCTION

Adriamycin and daunomycin are anthracycline antibiotics often used in cancer therapy [1]. Although their primary function is attributed to their ability to intercalate into DNA disrupting DNA and RNA synthesis [2], other modes of action involving free radical mechanisms which may be lethal to cells are also possible [3]. For instance, authors in [4] have shown that DNA strand scission occurs when adriamycin is first reduced and subsequently allowed to intercalate into DNA. Authors in [5] have also shown the formation of free hydroxyl radicals upon reductive activation of adriamycin and daunomycin in aqueous solutions. The hydroxyl radical is a powerful oxidant and in biological systems there is no known specific enzyme for its removal. Instead, cells rely on the removal of the hydroxyl radical precursors, hydrogen peroxide and the superoxide anion radical. Several reports have confirmed the formation of the superoxide anion radical in incubations of cellular components with anthracycline antibiotics [6–8].

The photosensitizing effects of adriamycin and daunomycin are of interest in connection with our work. Light sensitized viral and bacterial death in the presence of daunomycin was reported in [9] and [10]. Recently, authors [11] have shown the photodegradation of DNA by daunomycin and attributed this degradation to the attack on DNA by hydroxyl radicals produced in the solutions. However, in their study, they did not use classical hydroxyl radical scavengers, e.g., formate or ethanol [12–14], to unambiguously prove the direct formation of hydroxyl radicals in their photoirradiated DNA solutions.

An understanding of the photosensitizing properties of adriamycin and daunomycin is important because of a possible future application in combination therapy [11], similar to the photoir-

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radiation therapy with haematoporphyrin [15].

Here, evidence is given for the production of the superoxide anion radical in aqueous solutions of adriamycin and daunomycin irradiated with visible light. The technique of spin trapping, which consists of reacting short-lived radicals with a spin trap (a nitrone or nitroso compound) to give a longer lived ESR detectable nitroxide radical, was used to identify the formation of the superoxide anion radical in the adriamycin and daunomycin solutions.

2. EXPERIMENTAL

Adriamycin and daunomycin were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, National Institutes of Health.

The spin traps 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) and 2-methyl-2-nitrosopropane (MNP) were obtained from Aldrich. The 4-(*N*-methylpyridinium) *t*-butyl nitrone (4-MePyBN) was purchased from Spin Trap Co. (Canada). DMPO was purified as in [16], where solutions of DMPO were successively treated with activated charcoal until the disappearance of the free radical impurities. MNP and 4-MePyBN were used without further purification.

The photolyses were performed in situ at room temperature, in a standard quartz cell (60 × 10 × 0.25 mm) placed in the ESR cavity, using a Schoeffel 1000-W high-pressure Hg-Xe lamp with a Schoeffel grating monochromater. During the ESR measurements, the samples were irradiated continuously at a wavelength of 438 ± 10 nm. The ESR spectra were recorded on a Varian E-9 X-band spectrometer at 100 kHz magnetic field modulation.

3. RESULTS AND DISCUSSION

The photoexcitation of adriamycin or daunomycin in aqueous solutions (λ = 438 ± 10 nm) in the presence of the spin trap DMPO yielded an ESR spectrum consisting of a 1:2:2:1 quartet (fig.1). The equal nitrogen and hydrogen hyperfine coupling constants ($a_N = a_H^\beta = 14.9$ G) in this spectrum are characteristic of the hydroxyl spin adduct of DMPO [14,17].

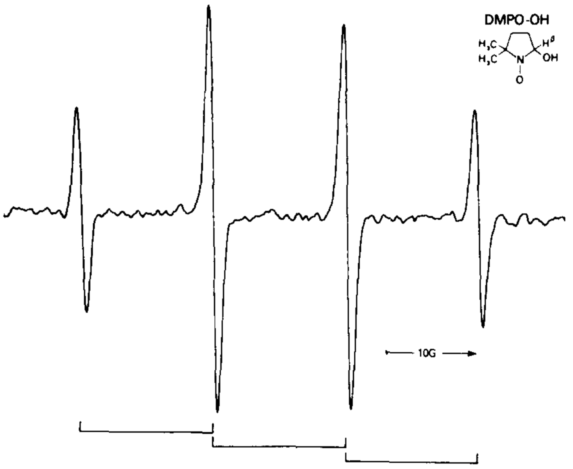

Fig.1. ESR spectrum of the DMPO-OH spin adduct formed during irradiation (438 ± 10 nm) of air-saturated adriamycin and daunomycin solutions.

In order to substantiate the direct generation of hydroxyl radicals by the photoexcited antibiotic compounds, the experiment was repeated in the presence of ethanol (1 M). Ethanol is known to react readily with hydroxyl radicals to give the α-hydroxyethyl radical which could be subsequently trapped by DMPO and identified [14]. However, we were unable to detect this spin adduct. This result indicates that hydroxyl radicals were not generated photochemically. Fig.2 shows the intensity of the DMPO-OH ESR spectrum *vs* time during the photoirradiation (λ = 438 ± 10 nm) of air-saturated and air-free (N_2-saturated) adriamycin (3×10^{-3} M) and daunomycin (1×10^{-5} M) solutions with and without ethanol. The DMPO-OH spin adduct is only formed in the presence of oxygen. Similar results were obtained when 1×10^{-5} M adriamycin and 3×10^{-3} M daunomycin solutions were used. Alternatively, the formation of hydroxyl radicals was tested by methyl radical release from dimethylsulfoxide (DMSO) [18]. Solutions of adriamycin and daunomycin (3×10^{-3} M) were photoirradiated (λ = 438 ± 10 nm) in the presence of DMSO (0.2 M) and MNP (1 mg/ml). Since the ESR spectrum of the methyl spin adduct of MNP was not observed, it is also concluded that hydroxyl radicals are not formed in the photochemical reactions.

These results indicate that the DMPO-OH spin adduct was not formed by the direct addition of hydroxyl radicals to DMPO but by an alternative

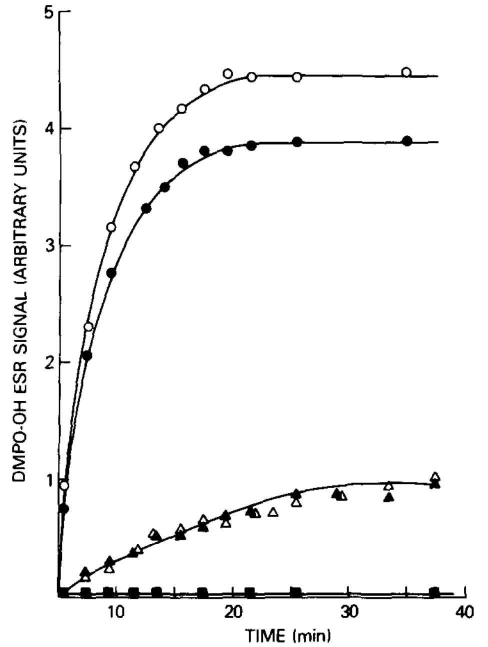

Fig.2. DMPO-OH ESR signal intensity *vs* time during irradiation (438 ± 10 nm) of adriamycin (3×10^{-3} M) and daunomycin (1×10^{-5} M) solutions. Air-saturated solutions: (○) adriamycin, no ethanol; (●) adriamycin, ethanol (1 M); (△) daunomycin, no ethanol; (▲) daunomycin, ethanol (1 M). Nitrogen-saturated solutions: (■) adriamycin or daunomycin, with or without ethanol.

route. It is known that the spin adduct of DMPO and the superoxide anion radical, DMPO-O_2^-, decomposes into the DMPO-OH adduct [17] or releases hydroxyl radicals by another minor reaction pathway [19].

Direct evidence for the light-induced production of the superoxide anion radical in adriamycin and daunomycin solutions can be obtained using the nitrone spin trap 4-MePyBN. The 4-MePyBN-O_2^- spin adduct has half lives of 83, 78 and 65 s at pH 5.5, 7.0 and 8.0, respectively [20]. It is easily observed by ESR and has characteristic hyperfine coupling constants ($a_N = 13.78$ G and $a_H^\beta = 1.65$ G) different from the 4-MePyBN-OH spin adduct ($a_N = 14.70$ G and $a_H^\beta = 1.45$ G) [21]. Fig.3 shows the ESR spectrum obtained during the photoirradiation ($\lambda = 438 \pm 10$ nm) of an adriamycin solution (3×10^{-3} M) containing 0.1 M 4-MePyBN at pH 5.5. The nitrogen and hydrogen hyperfine coupling constants, $a_N = 13.80$ G and $a_H^\beta = 1.58$ G, are consistent with the reported values for the 4-MePyBN-O_2^- ESR spectrum [20]. An ESR spectrum identical to the one shown in fig.3 was obtained when daunomycin was used instead of adriamycin. Finally, the photoirradiation of air-free (N_2-saturated) adriamycin and daunomycin solutions did not produce the DMPO-OH (fig.2), 4-MePyBN-O_2^- and 4-MePyBN-OH spin adducts. This is further evidence that the radical species observed in fig.1 originates from molecular oxygen and not via other pathways leading to the direct formation of hydroxyl radicals. A minor component in fig.3 was also observed and in the absence of light (after photoirradiation for 3 min) this signal increased with time while the ESR signal of the 4-MePyBN-O_2^- disappeared. The new ESR spectrum is similar to the ESR spectrum of the 4-MePyBN-OH spin adduct [21]. In the absence of a direct hydroxy radical production in the photoirradiated adriamycin and daunomycin solutions, the exact mechanism for the formation of a 4-MePyBN-OH spin adduct is unknown. However, hydroxyl radicals can be formed by the disproportionation of the superoxide anion radical in the presence of trace amounts of ferric salts [16]. Although precautions were taken using the iron chelating agent diethylenetriaminepentaacetic acid (DETAPAC, 1 mM) to inhibit an indirect produc-

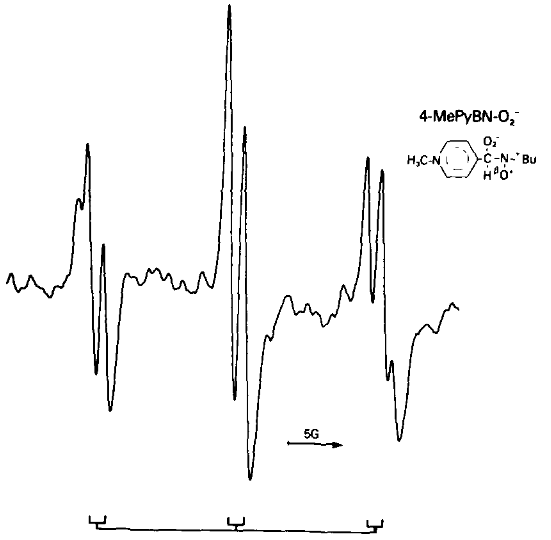

Fig.3. ESR spectrum of the 4-MePyBN-O_2^- spin adduct formed in photoirradiated (438 ± 10 nm) adriamycin solutions at pH 5.5.

tion of hydroxyl radicals from the superoxide anion radical, it is virtually impossible to eliminate all traces of metal ions from the solutions. Also, a small fraction of hydroxyl radicals could be formed from conceivable minor reaction pathways in the decomposition of 4-MePyBN-O_2^{-} similar to other nitrone spin traps [19].

Possible photochemical pathways that generate the superoxide anion radical are:

(i) photoionization, in which an electron is ejected from adriamycin or daunomycin and the resulting hydrated electron reacts with dissolved oxygen to form $O_2^{\cdot -}$;

(ii) the direct transfer of an electron from the excited states of the drugs to oxygen in solution.

Evidence against the photoionization mechanisms was obtained as follows: solutions of adriamycin and daunomycin were irradiated with light (λ = 438 ± 10 nm) in the presence of glycine and MNP. Hydrated electrons rapidly deaminate amino acids and the deaminated radicals are trapped by MNP yielding characteristic ESR spectra [22]. The ESR spectrum of the spin adduct of the deamination radical of glycine was not observed. Furthermore, the oxygen quenching of the emission spectra in fluorescence studies carried out in air-free, air-saturated and oxygen-saturated solutions of adriamycin and daunomycin, suggest a direct interaction between the drugs and oxygen. Fig.4 shows the fluorescence spectrum obtained from adriamycin solutions (1×10^{-5} M) which were nitrogen-saturated, air-saturated and oxygen-saturated. Because the oxygen concentration in the air-saturated solutions (280 μM) is much larger than the adriamycin concentration, identical spectra were obtained in the air-saturated and oxygen-saturated solutions. Similar fluorescence spectra to those in fig.4 were obtained in analogous experiments containing daunomycin.

The negative results obtained in the experiments designed to detect photoionization in addition to the results obtained in the fluorescence experiments, suggest that a direct electron transfer to oxygen from the electronically excited drugs or from their anion radicals ([drug] > 10^{-3} M) [23], is the most probable mechanism for the superoxide anion radical production in the photoirradiated adriamycin and daunomycin solutions.

It should be noted that the photoexcitation study of daunomycin in the presence of DNA was reported recently [11]. In that case, a DMPO-OH adduct was also formed. However, the mechanism of formation appears to be different since the presence of molecular oxygen in these studies was not required.

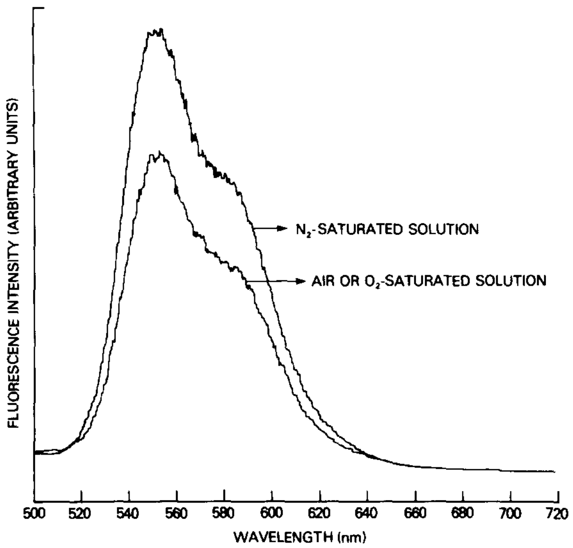

Fig.4. Fluorescence spectra of air-free (N_2-saturated), air-saturated and oxygen-saturated adriamycin solutions.

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