THE PRODUCTION OF HYDROXYL RADICAL BY TALLYSOMYCIN AND COPPER(II)

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1. Introduction

Tallysomycin is an antitumor antibiotic that is structurally related to bleomycin. The mechanism of action of tallysomycin appears to be similar to that of bleomycin. Bleomycin induces strand scission of purified isolated DNA [1–5]. Bleomycin has also been shown to produce breakage of intracellular DNA [5,6]. Iron(II) appears to be associated with the mechanism of action of bleomycin. The following model for the action of bleomycin has been proposed [1]. Bleomycin can bind to DNA. Fe(II) can then attach to the bleomycin to form a ternary complex. This complex, in the presence of oxygen, can then produce a species which degrades DNA. We have recently shown that bleomycin and Fe(II) produces the hydroxyl radical [7]. Because of the high reactivity of 'OH, it is likely that this radical is responsible for the toxicity of bleomycin.

Tallysomycin, a third generation bleomycin analogue, has been found to be more potent than bleomycin in the Walker 256 carcinoma and the P-388 leukemia tumor systems [8], as well as in a variety of bacteria and fungi [9]. Antitumor activity has also been observed for other tumor systems [9].

The mechanism of action of tallysomycin appears to be similar to that of bleomycin in that it produces strand breaks in purified isolated DNA and intracellular DNA similar to bleomycin [5]. However, the increased efficacy of tallysomycin to bleomycin could not be correlated with the breakage of DNA in vitro [5]. It was also observed that the concentration of ferrous ion appeared not to be a factor in the greater breakage of DNA observed for bleomycin over that of tallysomycin.

Using the technique of spin trapping we have observed the production of hydroxyl radical by tallysomycin and copper(II).

2. Materials and methods

2.1. *Reagents*

Tallysomycin was a gift from Dr William T. Bradner of Bristol Labs, East Syracuse, NY, lot no. 87F-11. The tallysomycin was dissolved in 50 mM potassium phosphate buffer (pH 7.5) immediately before use. The spin trap 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) was purchased from Aldrich Chem. Co., Milwaukee, WI. The colored impurity was removed by filtration with decolorizing charcoal using ~10 parts water to 1 part DMPO [10]. The purified DMPO solution was frozen until used. All other chemicals were reagent grade.

2.2. *Methods*

All additions were made to 50 mM potassium phosphate buffer (pH 7.5). All reaction mixtures contained ~70 mM DMPO. Electron spin resonance (EPR) measurements were carried out using a Varian E-9 spectrometer with an aqueous sample cell at room temperature using standard procedures.

3. Results

DMPO, 70 mM in phosphate buffer, produced no signal. When either 180 μM tallysomycin or 500 μM copper(II) chloride was added to the buffer in the presence of 70 mM DMPO, no signal was observed.

When 180 μM tallysomycin, 70 mM DMPO and 100 μM Fe(II) chloride was added to buffer no signal appeared. (A similar mixture with bleomycin results in the hydroxyl spin adduct signal of DMPO [7].) However, when 180 μM tallysomycin, 70 mM DMPO and 180 μM Cu(II) was added to buffer the signal shown in fig.1 appeared. ($A_N = A_\beta^H = 15.0$ G, $g = 2.006$). This 1:2:2:1 quartet signal has been assigned to the hydroxyl spin adduct of DMPO [11,12]. When the Cu(II) in the above experiment was first chelated with a 5-fold excess of EDTA before addition to the mixture, the signal was abolished. When superoxide dismutase (80 units/ml) or catalase (1000 units/ml) was included in the above mixture no change in signal was observed. Also bubbling the above solution with oxygen for 10 min before the addition of Cu(II) (the last addition to the mixture) resulted in no significant change in signal intensity.

When a solution of 500 μM tallysomycin, 70 mM DMPO and 16 mM Cu(II) was prepared in phosphate buffer a spectrum similar to that in fig.1 was obtained. However, when this mixture was bubbled with oxygen for 5 min before the addition of the Cu(II) the signal of fig.2 resulted. This spectrum is the result of two spin adducts of DMPO being present, one with spectral parameter of $A_N = A_\beta^H = 15.0$ G, $g = 2.006$ and another with parameters of $A_N = 16.1$ G, $A_\beta^H = 24.0$ G and $g = 2.006$. In addition, the resulting solution had a slight milky-white appearance with a visible precipitate as opposed to the clear solution observed in the mixture described earlier.

4. Discussion

These results suggest that copper(II) may have a role in the mode of action of tallysomycin in contrast

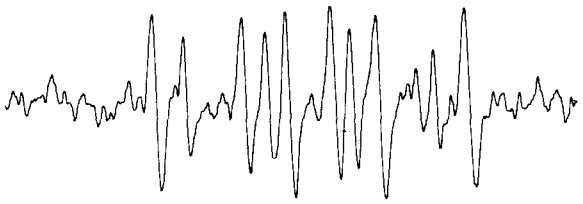

Fig.1. ESR spectra of the hydroxyl spin adduct of DMPO produced by a reaction mixture containing 180 μM tallysomycin, 70 mM DMPO, 180 μM copper(II) chloride in 50 mM phosphate buffer (pH 7.5). (See text for spectral parameters.)

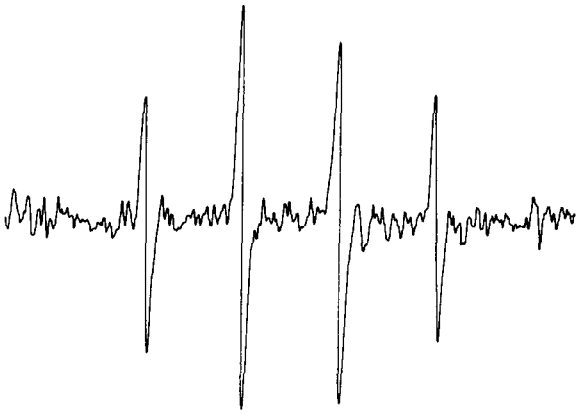

Fig.2. ESR spectra of the spin adducts of DMPO produced by a reaction mixture containing 500 μM tallysomycin, 70 mM DMPO, and 16 mM copper(II) chloride in 50 mM phosphate buffer. The solution was bubbled with oxygen for 5 min before the final addition of Cu(II) was made. The spin adducts observed appear to be the result of trapping hydroxyl radical and a carbon-centered free radical. (See text for spectral parameters.)

to the apparent role of iron(II) for bleomycin. It has been shown [13] that there are apparently two metal binding sites in the tallysomycin molecule and that copper is chosen over iron to occupy the second site. The last experiment described above suggests that oxygen may also be involved in the mode of action. The results of this experiment can be rationalized as follows: The additional oxygen present apparently increased the reactivity of tallysomycin such that it is essentially self destructive by a radical process. The large splitting constants observed in the additional signal present in fig.2 suggests a carbon-centered radical as opposed to an oxygen-centered radical [14]. Thus, we could have trapped radical fragments of the tallysomycin generated during its self destruction.

Since neither superoxide dismutase nor catalase affected the signal intensity, it appears that neither free superoxide nor hydrogen peroxide is involved in the mechanism of action. Apparently, ·OH can be produced by tallysomycin without these intermediates.

The results of this report suggest that, indeed, tallysomycin may function via a radical process and that copper(II) may have a role in its mode of action. Additional studies need to be done to elucidate further any possible role of copper(II) so that therapies utilizing

tallysomycin can be designed to reflect the possible need for copper.

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