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One-electron reduction of an anthracycline antibiotic carminomycin by a human erythrocyte redox chain

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Human erythrocyte membranes catalyse the NAD(P)H-dependent generation of the semiquinone of an adriamycin-type antibiotic carminomycin under anaerobic conditions. The maximal yield of the antibiotic radical is about 4-fold higher in the presence of NADPH than of NADH. The possible significance of the antibiotic reduction to the semiquinone by a human erythrocyte membrane redox chain for the clinical usage of these antibiotics is discussed.

NAD(P)H-dependent reduction; Erythrocyte membrane; Anthracycline antibiotic; Free radical; ESR

1. INTRODUCTION

Anthracycline antibiotics are widely used in cancer treatment due to their cytotoxicity toward tumors. It has been believed that binding to DNA is the main mechanism underlying the cytotoxic action of anthracycline antibiotics [1]. However, evidence has also been accumulated in favor of the view that the cytotoxic effect of these compounds involves the generation of antibiotic free radicals and active oxygen species during the course of redox activation [2]. It is known that anthracycline antibiotics can undergo reduction to semiquinone radicals by liver microsomal [3] and nuclear [4,5] redox chains in the presence of NADPH or NADH as electron donors. It seemed interesting to investigate whether a similar effect can take place in the erythrocyte membrane which is known to contain a highly active redox chain [6], since 30–50% of the pool of anthracycline antibiotics circulating in blood is associated with erythrocytes [7]. Carminomycin (fig.1) was chosen for these studies rather than adriamycin because the radical yield is much higher (at least 20-fold) for carminomycin than for adriamycin [5].

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2. MATERIALS AND METHODS

Human erythrocyte membranes were isolated by hypotonic hemolysis in 20 mM sodium phosphate buffer and serial washings with 20, 10 and 5 mM phosphate, pH 7.4. The membranes were quickly frozen in dry ice-acetone and stored at −25°C until use. Membrane protein concentration was estimated by the Lowry method.

For measurements of semiquinone free radical generation, membranes were suspended in 0.2 M potassium phosphate buffer, pH 7.0, deaerated by bubbling argon and supplied under anaerobic conditions with 1 mM carminomycin and 5 mM NADPH or NADH (final concentrations). The mixture was transferred to a stoppered flat quartz cell for ESR spectroscopy and measured in a Varian E-2 X-band ESR spectrometer at various time intervals. The temperature of the sample was maintained at 25°C. Measurement conditions

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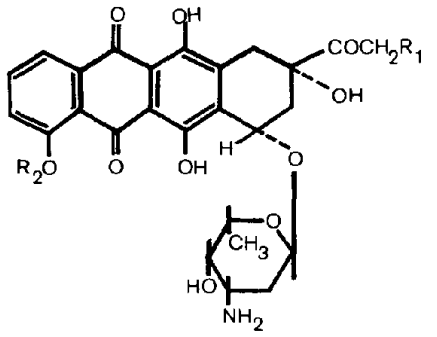

Adriamycin $R_1 = OH$ $R_2 = CH_3$

Carminomycin $R_1 = R_2 = H$

Fig.1. Structural formula of adriamycin and carminomycin.

were: modulation frequency, 100 kHz; modulation amplitude, 5 G; microwave power, 10 mW; scanning rate, 12.5 G/min; time constant, 0.3 s.

3. RESULTS AND DISCUSSION

Anaerobic incubation of human erythrocyte membranes in the presence of carminomycin and NADPH or NADH resulted in the appearance of an ESR spectrum of the semiquinone free radical of the antibiotic (not shown) similar to that observed previously for microsomal and nuclear preparations from rat liver and hepatoma 22a [5]. The kinetics of the free radical formation were rather complex (fig.2) and resemble those in [5]. The maximal height of the signal increased only slightly with increasing membrane concentration (cf. table 1). The maximal free radical concentration was about 4-times higher with NADPH than NADH as electron donor (table 1) as in the case of microsomes or nuclei from rat liver, in contrast to tumor nuclei which generate the carminomycin radical equally effectively in the presence of NADPH or NADH [5].

These results demonstrate that the redox chain of the human erythrocyte plasma membrane is capable of univalent reduction of anthracycline antibiotics to a semiquinone free radical. This finding may have important implications for the therapeutic use of those drugs, inasmuch as the resultant semiquinones can react with dioxygen to produce superoxide at a high rate [8]. Superoxide

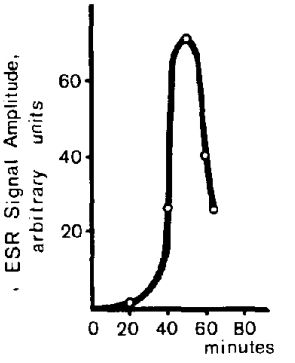

Fig.2. Kinetics of carminomycin semiquinone radical formation and decay in the presence of NADPH and human erythrocyte membranes (50 μg protein/ml).

(or its products) is capable of inducing oxidation and inter- as well as intramolecular crosslinking of hemoglobin [9] and of damaging the erythrocyte membrane [10]. Incidentally, whereas cancer usually results in a decrease in the erythrocyte count in blood, the concentration of anthracycline antibiotics in red blood cells in tumor-bearing animals is increased as compared with controls under conditions of equal antibiotic concentration in whole blood [7]. Hence, red blood cell damage may be one of the side effects of anthracycline therapy.

Recently, encapsulation of adriamycin in human erythrocytes has been suggested [11] as a new therapeutic approach allowing the slow release of a drug in the circulation. In our opinion, the ability

Table 1

The maximal yield of the carminomycin semiquinone during anaerobic NAD(P)H-dependent reduction of the antibiotic by human membranes

Electron donor	Membrane protein concentration (mg/ml)	
	0.5	0.05
NADPH (5 mM)	100 ± 3	70 ± 3
NADH (5 mM)	23 ± 4	16[a]

[a] Value obtained at the longest observation time (1 h); the true maximum may be slightly higher

For experimental conditions see section 2. Data are given in arbitrary units as means ± SD for 3 measurements, 100 corresponding to a radical concentration of 25 μM

of the human erythrocyte membrane redox chain to generate anthracycline antibiotic semiquinones must be taken into account in the clinical evaluation of such a method.

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