

Mitomycin C-induced deoxyribose degradation inhibited by superoxide dismutase

A reaction involving iron, hydroxyl and semiquinone radicals

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Mitomycin C stimulates deoxyribose degradation with the release of thiobarbituric acid-reactive material under conditions of low oxygen concentration. This damage is inhibited by scavengers of the hydroxyl radical, iron chelators and the specific proteins catalase and superoxide dismutase. The reactive radical species appears to arise from a Fenton-type sequence in which iron is reduced by the mitomycin C semiquinone radical.

Semiquinone radical

*Antitumor antibiotic
Deoxyribose degradation*

*Hydroxyl radical
TBA-reactivity*

Superoxide dismutase

1. INTRODUCTION

Mitomycin C is an antitumour antibiotic isolated from cultures of *Streptomyces caespitosus* [1]. Its anticancer properties have been attributed to functions of its aziridine, carbamate and quinone structures [2]. Like most antitumour antibiotics, mitomycin C binds to DNA. When reductively activated it can alkylate, cross-link and cause strand scissions in the DNA molecule [3-5].

Mitomycin C is used in cancer chemotherapy regimens, since it has activity against some solid tumours, leukaemias and adenocarcinomas [6,7]. The most serious toxicity of mitomycin C therapy is, however, a delayed cumulative myelosuppression [6].

Authors in [8] showed that reduction of the quinone group in mitomycin, by an NADPH-dependent microsomal enzyme system, resulted in the formation of a drug semiquinone radical. They further observed that sulphite oxidation, resulting from mitomycin C-stimulated microsomal incubations, was inhibited by the enzyme superoxide

dismutase (SOD). It has been suggested that the mitomycin C-induced degradation of DNA observed both in vivo and in vitro [9,10] might result from the generation of active oxygen species [5,11,12], possibly the hydroxyl radical [5,11].

Since mitomycin C has been shown to be more toxic to tumour cells growing in culture under hypoxic conditions [13], the following study was undertaken to examine the mechanisms by which mitomycin C, under conditions of low O₂ concentration, can degrade 2'-deoxyribose in a reaction inhibited by superoxide dismutase.

2. METHODS AND MATERIALS

2-Deoxy-D-ribose, diethylenetriaminepenta-acetic acid (DETAPAC), NADPH, hypoxanthine, mitomycin C, albumin (human, fatty acid-free), catalase (bovine liver, thymol-free), ferredoxin reductase, superoxide dismutase (bovine erythrocyte) and xanthine oxidase grade I (28.1 units/ml) were obtained from Sigma. Desferrioxamine was from Ciba-Geigy. All other chemicals were of the

highest grades available from BDH. Units of enzyme activity were as defined in the Sigma catalogue.

2.1. Mitomycin C-induced deoxyribose degradation

The following reagents were added to new clean glass tubes (9×1.5 cm) were tight-fitting screw-caps: 0.2 ml deoxyribose (10 mM), 0.2 ml hypoxanthine as a saturated aqueous solution, 0.1 ml of mitomycin C (1 mM) and 0.1 ml phosphate saline buffer, pH 7.4 (0.024 M phosphate, 0.15 M NaCl). The tube contents were gassed with nitrogen for 1 min exactly, and then immediately tightly capped. To each reaction was carefully added, with minimal introduction of air, 0.1 ml of xanthine oxidase (0.7 enzyme units/ml). Samples were incubated at 37°C for 30 min. Reduction of mitomycin C was also achieved by adding 0.2 ml of NADPH (1.6 mM) instead of hypoxanthine and 0.1 ml ferredoxin reductase (2 mg/ml) to replace xanthine oxidase. Where indicated scavengers and inhibitors were added to the reaction mixture before gassing with nitrogen. Appropriate controls and blanks were included with each batch.

2.2. Development of thiobarbituric acid (TBA) reactivity

After incubation, 0.5 ml TBA (1%, w/v) in 0.05 M NaOH and 0.5 ml trichloroacetic acid (2.8%, w/v) were added to each tube, followed by heating at 100°C for 15 min. The absorption of resulting pink chromogen was read at 532 nm.

The results shown are means of 3 separate experiments with results differing by not more than 6%.

3. RESULTS

Mitomycin C induced degradation of deoxyribose to form TBA-reactive material in the presence of xanthine oxidase, under conditions of low O_2 concentration. This degradation showed a linear dose-dependence for added mitomycin C in the range 0.1–1.0 mM (not shown). Under air, mitomycin C did not stimulate deoxyribose degradation above that seen with xanthine oxidase and hypoxanthine alone giving an absorbance at 532 nm of which 0.19 responded to inhibitors and oxygen radical scavengers as in [14].

Addition of a variety of hydroxyl radical scavengers to the mitomycin C-dependent reaction at low O_2 concentrations suggested that the OH^\cdot radical, or a species with similar reactivity, was responsible for deoxyribose degradation (table 1). Urea, which is not an OH^\cdot scavenger, was included as a control. The metal chelators desfer-

Table 1

Mitomycin C-induced deoxyribose degradation in the presence of xanthine oxidase. The effect of proteins, metal chelators and OH^\cdot scavengers

	Deoxyribose degradation under low concentrations of air as TBA-reactivity/0.5 h	
	$A_{532\text{ nm}}$	Inhibition (%)
Blank 1 (XOD + HXN + deoxyribose only)	0.100	
Blank 2 (mitomycin C + deoxyribose)	0.053	
Control (deoxyribose + mitomycin + XOD + HXN)	0.524	
+ Catalase 0.05 mg/ml	0.119	77
+ Catalase 0.02 mg/ml	0.131	75
+ Catalase 0.05 mg/ml heat-denatured	0.556	0
+ SOD 0.05 mg/ml	0.302	42
+ SOD 0.02 mg/ml	0.406	23
+ SOD 0.05 mg/ml heat-denatured	0.362	31
+ Albumin 0.05 mg/ml	0.520	1
+ Albumin 0.02 mg/ml	0.597	0
+ Albumin 0.05 mg/ml heat-denatured	0.584	0
+ EDTA 0.285 mM	0.642	0
+ Desferrioxamine 0.285 mM	0.130	75
+ DETAPAC 0.285 mM	0.091	83
+ Formate 14.3 mM	0.338	36
+ Benzoate 14.3 mM	0.309	41
+ Butan-1-ol 14.3 mM	0.371	29
+ Mannitol 14.3 mM	0.141	73
+ Thiourea 1.4 mM	0.157	70
+ Urea 1.4 mM	0.492	6

Concentrations shown are final reaction concentrations. % inhibition was calculated after subtraction of blank values. XOD, xanthine oxidase; HXN, hypoxanthine. Proteins were heated for 5 min at 100°C to denature them

Table 2

Mitomycin C-induced deoxyribose degradation in the presence of xanthine oxidase: the effect of SOD under different concentrations of air

	Air in reaction mixture (%)	Deoxyribose degradation/0.5 h as TBA-reactivity	
		$A_{532 \text{ nm}}$	Inhibition (%)
Blank 1 (XOD + HXN + deoxyribose only)	0	0.084	
Blank 2 (deoxyribose + mitomycin C only)	0	0.053	
Control 1 (deoxyribose + mitomycin C + XOD + HXN)	0	0.596	
+ SOD 0.05 mg/ml	0	0.546	8
Control 1	5	0.393	
+ SOD 0.05 mg/ml	5	0.263	33
Control 1	10	0.306	
+ SOD 0.05 mg/ml	10	0.184	40
Control 1	20	0.276	
+ SOD 0.05 mg/ml	20	0.106	62

Final reaction concentrations are shown. Air mixtures were prepared as in [15]. SOD, superoxide dismutase; XOD, xanthine oxidase; HXN, hypoxanthine. The blank values at each % concentration of air were the same as those shown at 0%

rioxamine and DETAPAC strongly inhibited deoxyribose degradation, whereas EDTA stimulated it (table 1). Both catalase and superoxide dismutase at a final reaction concentration of 0.02 mg/ml inhibited mitomycin C-induced deoxyribose degradation (table 1). Albumin served as a protein control for these enzymes. Heat denaturation of the proteins destroyed the protective activity of catalase but not that of SOD (table 1). The inhibitory activity of SOD was dependent on the percentage concentration of air present in the reaction tube. As the concentration of air increased, the degradation of deoxyribose decreased. This was paralleled by an increase in the ability of SOD to inhibit the reaction (table 2). Addition of hydrogen peroxide to the reaction under anaerobic conditions greatly enhanced deoxyribose degradation which could be inhibited by catalase and desferrioxamine (table 3).

Ferredoxin reductase, in the presence of NADPH, could substitute for xanthine oxidase in the mitomycin-induced deoxyribose degradation reaction. This degradation was inhibited by catalase and desferrioxamine but not by SOD (not shown).

Table 3

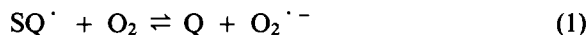
Mitomycin C-induced deoxyribose degradation in the presence of xanthine oxidase under anaerobic conditions: the effect of added hydrogen peroxide

	Deoxyribose degradation/0.5 h as TBA-reactivity	
	$A_{532 \text{ nm}}$	Inhibition %
Blank 1 (XOD + HXN + deoxyribose only)	0.100	
Blank 2 (deoxyribose + mitomycin C)	0.015	
Control 1 (deoxyribose + mitomycin C + XOX + HXN)	0.524	
Control 2 (as above + H_2O_2 0.0143 mM)	0.764	
Control 2	0.191	64
+ Catalase 0.05 mg/ml		
Control 2	0.170	68
+ Desferrioxamine 0.285 mM		

Final reaction concentrations are shown. XOD, xanthine oxidase; HXN, hypoxanthine; % inhibition was calculated after subtraction of blank values

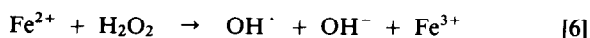
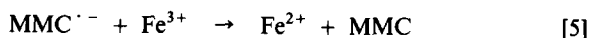
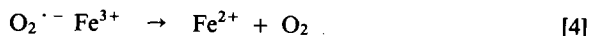
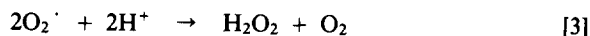
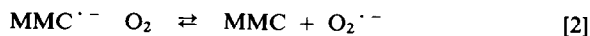
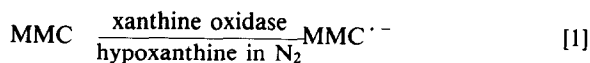
4. DISCUSSION

The antitumour antibiotic mitomycin C (MMC) is here shown to degrade deoxyribose when enzymically reduced to its semiquinone form (MMC^{•-}). The quine-group containing anthracycline antitumour antibiotics doxorubicin [15,16] and daunomycin (unpublished) have been shown to behave in a similar way. Mitomycin C-induced deoxyribose degradation was partly inhibited by several OH[•] radical scavengers and substantially blocked by the metal chelators desferrioxamine and DETAPAC. The specific active oxygen scavengers catalase and SOD also inhibited damage to deoxyribose. Inhibition by catalase, in this reaction, points to a direct involvement of hydrogen peroxide in the formation of a species able to degrade deoxyribose, probably the OH[•] radical. Inhibition by SOD, however, does not necessarily imply a direct involvement of superoxide radicals in deoxyribose degradation. Winterbourn has shown that superoxide dismutase can influence reactions involving quinones even though the superoxide radical does not directly participate in the reaction [17,18]. Dismutation of O₂^{•-} formed in a reaction between a semiquinone (SQ^{•-}) and oxygen will displace the reaction to the right, thereby influencing radical precursors of superoxide:



Inhibition by SOD of the reactions described here, when given concentrations of air are in the mixture, suggest a reaction mechanism similar to that in [17,18]. Heat-denaturation of SOD does not always destroy its protective activity [14], possibly because displaced copper ions can readily recombine, on cooling, to restore some dismutase activity.

In addition to xanthine oxidase [19], ferredoxin reductase [20] and glutathione reductase [21] have been used to reduce quinones in cell-free systems. Here, only xanthine oxidase and ferredoxin reductase reduced mitomycin C. Ferredoxin reductase, unlike xanthine oxidase, does not produce superoxide radicals in reaction with its substrate, although this would not account for the lack of inhibitory activity seen when SOD was added to the reaction.



Overall net reactions

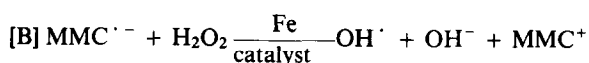
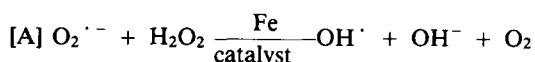


Fig.1

Deoxyribose degradation is stimulated by mitomycin C under conditions of low O₂ concentration. This is increased in the presence of hydrogen peroxide but inhibited by catalase, OH[•] scavengers, iron chelators and SOD. These results suggest reaction sequences similar to those summarised in fig. 1 take place in which reaction (A) predominates in air and reaction (B) is considerably more efficient at low oxygen concentrations. This latter finding may explain the preferential toxicity of mitomycin C to tumour cells under hypoxic conditions.

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