Reactions of the semiquinone free radicals of anti-tumour agents with oxygen and iron complexes

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The rates of reaction of the semiquinone radicals of adriamycin and mitomycin C with oxygen and several iron (III) complexes have been determined. These rates have been used to explain enhanced free radical damage produced by semiquinone radicals in a model system and to assess the feasibility of these types of reactions occurring in hypoxic cells.

Antitumor drug *Mitomycin C* *Adriamycin* *Oxygen* *Iron complex* *Pulse radiolysis*

1. INTRODUCTION

Mitomycin C and adriamycin are antitumour, antibiotic quinones which are used extensively in the clinical treatment of a variety of cancers. Both drugs can be reduced by an NADPH-dependent microsomal enzyme system to form semiquinone free radicals (e.g. [1,2]) which may be important in the in vivo functions of the drugs (e.g. [3,4]). One-electron interactions between the quinones and complexed iron and oxygen are of current interest (e.g. [5,6]). This work reports measurements of the absolute rate constants for the reaction of the semiquinone free radicals with oxygen and several iron complexes. The feasibility of these radicals giving enhanced free radical damage under hypoxic conditions has also been assessed.

2. MATERIALS AND METHODS

EDTA, diethylenetriaminepentaacetic acid (DETAPAC), mitomycin C (MMC), adriamycin (ADR) and ATP were obtained from Sigma. Desferrioxamine was from Ciba-Geigy. Iron (III) chloride and iron (III) perchlorate were from Hopkins and Williams. All other reagents were of the highest grades available from B.D.H.

The simple iron (III) complexes of DETAPAC, EDTA, ATP and desferrioxamine were freshly prepared as stock solutions by the addition of 3 mM iron (III) perchlorate to 12 mM complexing agent in 50 mM phosphate buffer (pH 7.0). Aliquots of these stock solutions were then added to the quinone solutions. Comparison of the spectra of the quinone solutions before and after the addition of the iron complexes showed that there was no indication of any quinone-iron interaction. This is consistent with the large association constants for the iron (III) complexes (e.g. [7–9]). The iron (III)-adriamycin complex was prepared according to the method of Bachur et al. [10]. Briefly, a stock solution of iron (III)-adriamycin was prepared by the addition of 200 μM iron (III)chloride to 200 μM adriamycin in water. After the appearance of the purple colour due to the complex formation, aliquots of this solution were added to adriamycin in 50 mM phosphate buffer (pH 7.0).

Pulse-radiolysis experiments were conducted using the Paterson Laboratories linear accelerator facility [11]. The optical detection system consisted of a tungsten lamp, a Bausch-Lomb monochromator and an EMI 9558QA photomultiplier. Micro-cells with optical path lengths of 2.5 cm

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(vol. = 0.15 ml) were used throughout. The signals from the photomultiplier were recorded on a Tektronix 7612D programmable digitizer and analysed by a Hewlett-Packard 9836s computer.

3. RESULTS

On giving short pulses (5 ns) of radiation to an argon saturated solution containing sodium formate (100 mM) and quinone drug (100–180 μM) in phosphate buffer (50 mM, pH 7.0), the following reactions are initiated:

$$H_2O \longrightarrow e_{aq}^-,\ {}^{\cdot}OH,\ H^{\cdot},\ H_2O_2,\ H_2 \quad (1)$$

$${}^{\cdot}OH(H^{\cdot}) + HCOO^- \longrightarrow H_2O(H_2) + CO_2^{\cdot -} \quad (2)$$

$$e_{aq}^- (CO_2^{\cdot -}) + Q \longrightarrow Q^{\cdot -} + CO_2 \quad (3)$$

In the absence of oxygen or iron complexes, the semiquinone radicals decay relatively slowly via second order kinetics to form the hydroquinone. This process can be conveniently observed for the adriamycin semiquinone radicals at 720 nm [12] and for the mitomycin C semiquinone radicals at 510 nm [13]. In the presence of oxygen or a reactive iron (III) complex the decay of the semiquinone radicals is much faster due to:

$$Q^{\cdot -} + O_2 \longrightarrow Q + O_2^{\cdot -} \quad (4)$$

or

$$Q^{\cdot -} + Fe^{3+}X \longrightarrow Q + Fe^{2+}X \quad (5)$$

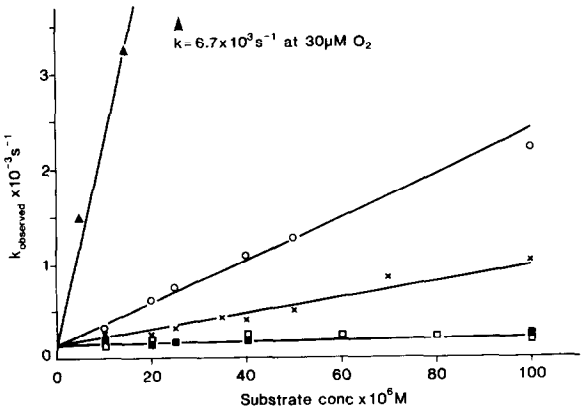

Fig.1. Observed reaction rates of mitomycin C semiquinone radicals with oxygen and iron (III) complexes. ▲, oxygen; ○, iron (III) DETAPAC; ×, iron (III) EDTA; ■, iron (III) desferrioxamine; □, iron (III) ATP.

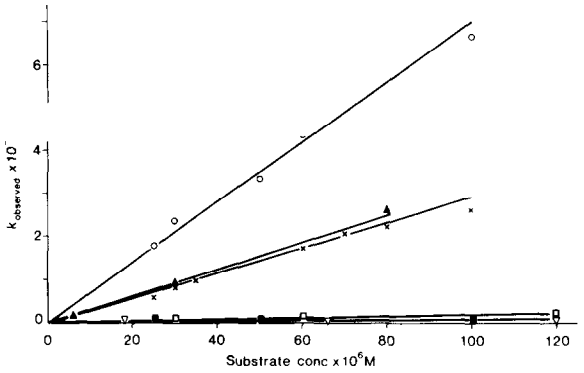

Fig.2. Observed reaction rates of adriamycin semiquinone radicals with oxygen and iron (III) complexes. ▲, Oxygen; ○, iron (III) DETAPAC; ×, iron (III) EDTA; ■, iron (III) desferrioxamine; □, iron (III) ATP; ▽, iron (III) adriamycin.

Table 1

Bimolecular rate constants for the reactions of mitomycin C and adriamycin semiquinone radicals with oxygen and iron III complexes

Substrate	Bimolecular rate constant ($M^{-1} \cdot s^{-1}$)	
	Mitomycin	Adriamycin
Oxygen	$2.2 \pm 0.2 \times 10^8$	$3.0 \pm 0.2 \times 10^8$
Ferric desferrioxamine	$<6 \times 10^4$	$<4 \times 10^4$
Ferric DETAPAC	$2.4 \pm 0.2 \times 10^7$	$7.0 \pm 0.3 \times 10^8$
Ferric EDTA	$9.0 \pm 0.8 \times 10^6$	$2.8 \pm 0.2 \times 10^8$
Ferric ATP	$<6 \times 10^4$	$8 \pm 1.4 \times 10^6$
Ferric adriamycin	–	$<10^5$

The rate constants were calculated from the increase in the rate of decay of mitomycin C radicals at 510 nm and adriamycin radicals at 720 nm (see section 3)

The results for the above reactions are shown in fig.1,2. The rate constants obtained are listed in table 1.

4. DISCUSSION

It can be seen from table 1 that the semiquinone radicals of mitomycin C tend to react more slowly than those of adriamycin. This is illustrated by the reactions with iron (III) ATP, for whereas the

reaction with mitomycin C radicals could not be detected ($k < 6 \times 10^4\ M^{-1} \cdot s^{-1}$), the rate constant with adriamycin radicals is appreciable ($k = 8 \pm 1.4 \times 10^6\ M^{-1} \cdot s^{-1}$). The differences in the reactivities are not due to the one-electron reduction potentials of the quinone/semiquinone couples as these are very negative, E_7(MMC/MMC$^{\cdot -}$) = −310 mV (unpublished) and E_7 (ADR/ADR$^{\cdot -}$) = −328 mV [12], but rather must be a reflection of the structures of the quinone radicals and the steric hindrance of the reactions. Table 1 also shows that the rate constants depend strongly on the nature of the iron (III) complex, for similar reasons.

The following scheme has been proposed [5] to explain the mitomycin C induced degradation of deoxyribose:

$$Q \xrightarrow{E} Q^{\cdot -} \quad (6)$$

$$O_2 \xrightarrow{E} O_2^{\cdot -} \quad (7)$$

$$2O_2^{\cdot -} + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (8)$$

$$Q^{\cdot -} + O_2 \rightleftharpoons Q + O_2^{\cdot -} \quad (9)$$

$$Q^{\cdot -} + Fe^{3+}X \longrightarrow Q + Fe^{2+}X \quad (5)$$

$$O_2^{\cdot -} + Fe^{3+}X \longrightarrow O_2 + Fe^{2+}X \quad (10)$$

$$H_2O_2 + Fe^{2+}X \longrightarrow {}^{\cdot}OH + OH^- + Fe^{3+}X \quad (11)$$

where E represents enzymatic reduction.

It has been demonstrated that deoxyribose degradation by ·OH radicals is stimulated under low oxygen concentrations but inhibited by high oxygen concentrations and it has been suggested that these effects may explain the preferential toxicity of mitomycin C towards cells incubated under hypoxic conditions.

The one-electron reduction potential of the oxygen couple, E_7 ($O_2/O_2^{\cdot -}$) is −155 mV (for [O_2] = 1 M [14]) and hence from the reduction potentials given above it can be shown that the equilibrium constant for reaction (9) is 417 and 840 for mitomycin C and adriamycin, respectively. Thus, the probability of $Q^{\cdot -}$ reactivity with oxygen or Fe^{3+} depends simply on the forward rate constants of reactions (5) and (9). The results of this study show that in general the oxygen concentration has to be less than that of the iron (III) complexes to form any significant amount of Fe^{2+} from reaction (5). However $O_2^{\cdot -}$ radicals have to be produced in order for H_2O_2 to be formed to give reaction (11).

The enhanced production of ·OH radicals under hypoxic conditions could be explained simply by the relative rate constants of $O_2^{\cdot -}$ or $Q^{\cdot -}$ radicals with iron (III). The results presented here for reaction (5) show that the rate constants are at least an order of magnitude larger than those previously reported for the $O_2^{\cdot -}$ reaction [15]. Thus, under hypoxic conditions the semiquinone radicals should form more Fe(II) than under oxic conditions where only $O_2^{\cdot -}$ is produced (and hence the semiquinone radicals should form less H_2O_2 than the pure $O_2^{\cdot -}$). The limiting substrate under hypoxic conditions is therefore H_2O_2 and this is confirmed by the results of Gutteridge et al. [5] who showed that ·OH radical production under hypoxic conditions is further enhanced by the addition of excess H_2O_2.

The results presented here for iron (III) DETAPAC might appear to be contrary to that expected from the previous study [5] in which DETAPAC was found to inhibit the production of ·OH radicals. This anomaly can be explained if a further reaction is included in the scheme:

$$O_2^{\cdot -} + Fe^{2+}X \longrightarrow \text{oxidised iron} \quad (12)$$

Rate constants for these reactions have been measured [15], and for most iron (III) complexes, and for iron (III) DETAPAC in particular, the rate constants are several orders of magnitude higher than for the reduction of Fe(III), reaction (10). Thus, if iron (II) DETAPAC is produced by reaction (5) then the $O_2^{\cdot -}$ radicals, as they cannot react at any appreciable rate with iron (III) DETAPAC [15], will reoxidise the iron (II) back to iron (III) and hence deplete the iron (II) necessary to undergo reaction (11). The corresponding reaction with the mitomycin C radicals would be:

$$MMC^{\cdot -} + Fe^{2+}X \longrightarrow \text{MMC(hydroquinone)} + Fe^{3+}X \quad (13)$$

The two-electron reduction potential of the mitomycin C/hydroquinone couple has been estimated as −368 mV vs SCE [16]. Thus, using the above potential of the MMC$^{\cdot -}$/MMC couple of −310 mV it can be calculated that the MMC$^{\cdot -}$/MMC (hydroquinone) couple is +55 mV vs NHE. As the potentials of $Fe^{3+}X/Fe^{2+}X$ couples are generally more positive than +55 mV (e.g. [7,17]) it is unlikely that reaction (13) can occur.

Unfortunately, there is little information available on the amount of intracellular iron that can undergo reaction (11). However, on comparison with that in extracellular fluids [18], it is expected to be not much more than about 3 μM. As the study involving mitomycin C toxicity towards hypoxic cells used oxygen concentrations less than 10 ppm (i.e., $[O_2] < 0.01$ μM [19]), it is quite feasible that under these conditions the mitomycin C semiquinone radicals, if produced, can enhance radical damage by reducing intracellular iron. Further work is now in progress to quantify the results from model systems based on these rate constants and to assess the relevance of these studies in the in vivo situation.

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REFERENCES

[1] Handa, K. and Sato, S. (1974) Gann 66, 43–47.
[2] Bachur, N.R., Gordon, S.L., Gee, M.V. and Kon, H. (1979) Proc. Natl. Acad. Sci. USA 76, 956–957.
[3] Lown, J.W., Begleiter, A., Johnson, D. and Morgan, R. (1976) Can. J. Biochem. 54, 110–119.
[4] Bachur, N.R., Gordon, S.L. and Gee, M.V. (1978) Cancer Res. 31, 1745–1750.
[5] Gutteridge, J.M.C., Quinlan, G.J. and Wilkins, S. (1984) FEBS Lett. 167, 37–41.
[6] Eliot, H., Gianni, L. and Myers, C. (1984) Biochemistry 23, 928–936.
[7] Skochdopole, R. and Chaberek, S. (1959) J. Inorg. Nucl. Chem. 11, 222–233.
[8] Prelog, V. (1964) in: Iron Metabolism: An International Symposium (Gross, F. ed.) p.73, Springer, Berlin.
[9] Martell, A.E. and Sillen, L.G. (1971) Stability Constants of Metal-Ion Complexes, Suppl.1, Parts I and II. Special Publication 25, The Chem. Soc., London.
[10] Bachur, N.R., Friedman, R.D. and Hollerbeck, R.G. (1984) Cancer Chemother. Pharmacol. 12, 5–9.
[11] Keene, J.P. (1964) J. Sci. Inst. 41, 493–496.
[12] Land, E.J., Mukherjee, T., Swallow, A.J. and Bruce, J.M. (1983) Arch. Biochem. Biophys. 225, 116–121.
[13] Svingen, B.A. and Powis, G. (1981) Arch. Biochem. Biophys. 207, 119–126.
[14] Ilan, Y.A., Czapski, G. and Meisel, D. (1976) Biochim. Biophys. Acta 430, 209–225.
[15] Butler, J. and Halliwell, B. (1982) Arch. Biochem. Biophys. 218, 174–178.
[16] Rao, G.M., Begleiter, A., Lown, J.W. and Plambeck, J.A. (1977) J. Electrochem. Soc. 124, 199–202.
[17] Milazzo, G. and Caroli, S. (1978) Tables of Standard Electrode Potentials, Wiley Interscience, New York.
[18] Gutteridge, J.M.C., Rowley, D.A. and Halliwell, B. (1982) Biochem. J. 206, 605–609.
[19] Teicher, B.A., Lazo, J.S. and Sartorelli, A.C. (1981) Cancer Res. 41, 73–81.