Pergamon

Biochemical Pharmacology, Vol. 50, No. 1, pp. 75–82, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00112-3

EFFICIENT REDOX CYCLING OF NITROQUINOLINE BIOREDUCTIVE DRUGS DUE TO AEROBIC NITROREDUCTION IN CHINESE HAMSTER CELLS

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(*Received* 10 *November* 1994; *accepted* 11 *January* 1995)

Abstract—Nitroquinoline bioreductive drugs with 4-alkylamino substituents undergo one-electron reduction in mammalian cells, resulting in futile redox cycling due to oxidation of the nitro radical anion in aerobic cultures, and eventual reduction to the corresponding amines in the absence of oxygen. Rates of drug-induced oxygen consumption (R) due to redox cycling in cyanide-treated AA8 cell cultures were determined for 17 nitroquinolines. There was a linear dependence of log R on the one-electron reduction potential at pH 7 (E_7^1) with a slope of 7.1 V^{-1}, excluding compounds with substituents *ortho* to the nitro group. The latter had anomalously low rates of oxygen consumption relative to E_7^1, suggesting that interaction with the active site of nitroreductases is impeded sterically for such compounds. Absolute values of R (and the observed E_7^1 dependence) were well predicted by a simple kinetic model that used rates of net nitroreduction to the amines under anoxia as a measure of the rates of one-electron reduction in aerobic cells. This indicates that redox cycling of 4-alkylaminonitroquinolines occurs at high efficiency in aerobic cells, suggesting that there are no quantitatively significant fates of nitro radical anions in cells other than their reaction with oxygen (or their spontaneous disproportionation under hypoxia).

Key words: nitroquinolines; nitroreduction; oxygen consumption; redox cycling; cyanide

The mutagenicity and toxicity of aromatic nitro compounds, and their utility as therapeutic agents, are often a consequence of metabolic reduction of the nitro group [1]. Most enzymes capable of catalysing nitroreduction do so by one-electron reduction to form a nitro radical anion ($ArNO_2^{\bullet-}$) as the first intermediate in the reduction pathway [2]. Further reduction is considered to proceed by disproportionation of $ArNO_2^{\bullet-}$, in competition with its reoxidation by O_2 [2–4] (Fig. 1). The reaction of $ArNO_2^{\bullet-}$ with O_2 is responsible for generation of $O_2^{\bullet-}$, and hence toxicity due to reactive oxygen species [5, 6]. It is also responsible for the inhibition of net nitroreduction by O_2 [7, 8]; the further reduction to reactive nitroso and hydroxylamine derivatives in the absence of O_2 underlies the selective toxicity of nitro compounds to anaerobic microorganisms and to hypoxic tumour cells [9].

The above general features of one-electron nitroreduction are well-established, and there is ample evidence for the existence of nitro radical anions as nitroreduction intermediates in biological systems [2, 10, 11]. The kinetics of the relevant radical reactions have been studied thoroughly in dilute solution, particularly by pulse radiolysis [12], and the existence of a redox futile cycle in oxygenated cells is demonstrated by the increased consumption of O_2 and generation of H_2O_2 in the presence of nitro compounds [13, 14]. However, there does not appear to have been any attempt to test whether the simple kinetic model illustrated in Fig. 1 can account quantitatively for O_2 consumption induced by nitro compounds in whole cells. In particular, it is unclear

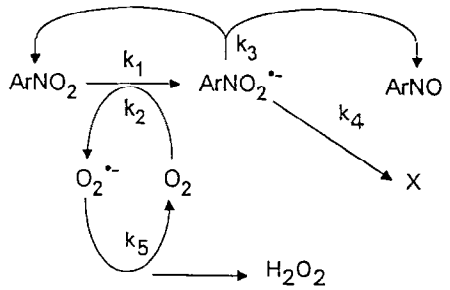

Fig. 1. Scheme for the one-electron reduction of nitro compounds and the operation of a futile redox cycle in the presence of oxygen. k_1 (etc.) are the rate constants for the indicated reactions.

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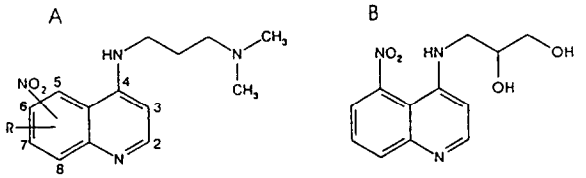
Table 1. Drug structures, E_7^1 values and rates of drug-induced oxygen consumption in cyanide-inhibited AA8 cells by 4-alkylaminonitroquinolines at 200 μM



No.	Structure formula; R =	E_7^1* (mV)[1]	Rate of drug-induced oxygen consumption† (nmol/min/10^6 cells)
1	A; 8-NO_2	−268 ± 11‡	2.739 ± 0.022
2	A; 2-Me 5-NO_2	−274 ± 11‡	1.754 ± 0.070
3	B	−276§	0.713 ± 0.121
4	A; 5-NO_2	−286 ± 11‡	1.470 ± 0.037‖
5	A; 6-Et 5-NO_2	−300 ± 11¶	0.223 ± 0.008
6	A; 8-OMe 5-NO_2	−310 ± 10‡	0.889 ± 0.060
7	A; 8-Me 5-NO_2	−316 ± 11‡	0.960 ± 0.067‖
8	A; 6-Me 5-NO_2	−319 ± 12‡	0.159 ± 0.021
9	A; 2,3-diMe 5-NO_2	−320§	0.477 ± 0.074
10	A; 7-NO_2	−323 ± 11‡	1.145 ± 0.107
11	A; 3,8-diMe 5-NO_2	−334 ± 10¶	0.182 ± 0.012‖
12	A; 6,8-diMe 5-NO_2	−335 ± 12¶	0.161 ± 0.022
13	A; 3-Me 5-NO_2	−369 ± 10‡	0.277 ± 0.032‖
14	A; 3,6-diMe 5-NO_2	−367 ± 8¶	<0.025
15	A; 6-NO_2	−392 ± 11‡	0.355 ± 0.044
16	A; 3-NO_2	−475 ± 12‡	0.033 ± 0.009
17	A; 8-NHMe 5-NO_2	−520 ± 12‡	0.049 ± 0.006‖

* Determined by pulse radiolysis in aqueous solutions containing 0.2 M propan-2-ol, 10 mM phosphate buffer, pH 7.0. Values are means ± SEM, N = 3–5.

† Mean ± range (compounds **2**, **5**, **6**, **8**, **10**, **13**, **15**) or SEM (N = 3).

‡ Data from Ref. 19, corrected assuming the E_7^1 for the redox indicator benzyl viologen to be −374 mV [20].

§ P. Wardman, personal communication. Cited with permission.

‖ Data from Ref. 21.

¶ R. F. Anderson, personal communication. Cited with permission.

whether oxygen might inhibit nitroreduction via other mechanisms (e.g. as a competing electron acceptor, or by inhibiting reductase expression relative to that in hypoxic cells), or whether there are other significant fates of nitro radicals in cells (represented by k_4 in Fig. 1). With regard to the latter, some nitro radical anions (e.g. those of 2-nitroimidazoles) appear to decay by an unidentified first-order process in dilute solution [3, 15], and reaction with thiols and cellular macromolecules has been suggested by several authors [7, 16, 17]. Contrary to this, it has been argued on thermodynamic grounds that the reduction of $ArNO_2^{\cdot-}$ by thiols is unlikely [12], and reduced glutathione (GSH) has been shown to have no effect on the lifetime of 5-nitrofuran radicals [18].

Recent studies in this laboratory with a series of 4-alkylaminonitroquinoline bioreductive drugs (see Table 1) have provided an opportunity to test the applicability of this simple kinetic model in cells. The compounds investigated are of biological interest because of the highly selective toxicity towards hypoxic cells (up to 60-fold) displayed by some members of the class [19, 22]. They are also useful model compounds for investigating metabolic nitroreduction since they are metabolized to the corresponding amines in high yield [21]. Further, an extensive series of analogues covering a wide range of E_7^1‡ is available. The rates of metabolism of the parent compounds have been measured by HPLC for several of these nitroquinolines in AA8 Chinese hamster cells under anoxic conditions [21]. The latter study demonstrated a linear relationship between rates of parent drug loss (under anoxia) and drug-stimulated O_2 consumption (under oxic conditions), consistent with the initial one-electron reduction step being rate-limiting for both. If the rates of loss of the nitro compounds under hypoxia are equal to the rates of the one-electron reduction under aerobic conditions, it is possible to test whether the observed rates of drug-stimulated O_2 consumption in intact cells can be accounted for by the operation of the cycle illustrated in Fig. 1.

In this study, we examined the rates of drug-induced O_2 consumption in cyanide-inhibited AA8

‡ Abbreviations: E_7^1, one-electron reduction potential(s) at pH 7.; $E_{1/2}$, polarographic half-wave reduction potential; HSC, hypoxia-selective cytotoxin; and R, rate of drug-induced cellular O_2 consumption.

cells for an extended series of 4-alkylaminonitroquinolines to test the above kinetic model. We also examined the dependence of drug-induced O_2 consumption on E_7^1 and demonstrated that there is a specific substituent effect on nitroreduction that is not mediated via changes in E_7^1.

MATERIALS AND METHODS

Compounds. All nitroquinolines were synthesized in the Auckland Cancer Research Laboratory, in most cases using published methods [19, 23]. The synthesis of compounds **3**, **5**, **9**, **11**, **12**, and **14** will be reported elsewhere.* All compounds had purities of > 98% based on chromatographic peak areas (monitored at 250 nm, bandwidth 80 nm) using minor modifications of HPLC conditions described previously [24]. Stock solutions of drugs were prepared in 50% aqueous ethanol and stored at −80°.

Cells. All studies used Chinese hamster AA8 cells demonstrated to be free of mycoplasma, which are known to give rise to cyanide-insensitive respiration [25], by cytochemical staining [26]. Cells were grown to 1–1.2 × 10^6/mL in spinner flasks as detailed previously [22], harvested by centrifugation and resuspended in culture medium [α-minimal essential medium containing 5% (v/v) heat-inactivated fetal bovine serum plus penicillin (100 IU/mL) and streptomycin (100 μg/mL)] containing 3 mM KCN [13], to a density of 10^7 cells/mL.

Drug-induced O_2 consumption in cyanide-inhibited cells. The method used for measuring drug-induced O_2 consumption in cyanide-inhibited cells is based on that of Biaglow *et al.* [13]. Aliquots (7 mL) of cell suspension were transferred to a glass respiration vial fitted with a ceramic spin bar and air-tight ceramic lid into which was inserted a Clark-type O_2 electrode [27] while carefully avoiding air bubbles. The vial was placed in a 37° water bath and stirred to equilibrate until the rate of oxygen consumption was linear. A small volume of drug in 50% ethanol was added to give a final concentration of 200 μM, unless otherwise stated, and the initial rate of drug-induced O_2 consumption was recorded. Before each experiment the O_2 electrode was calibrated using culture medium equilibrated with 20% O_2 at 37°, in which the concentration of dissolved O_2 was assumed to be 202 μM [28]. The rate of cellular O_2 consumption determined for AA8 cells (5 × 10^6 cells/mL in culture medium, no KCN) was 2.13 ± 0.15 nmol O_2/min/10^6 cells (mean ± SEM, N = 7). KCN (3 mM) resulted in 93% inhibition of the rate of cellular O_2 consumption.

Effect of KCN *on rates of nitroreduction.* Compound **7** (initial concentration 200 μM) was incubated in stirred AA8 cultures (7 mL at 10^7 cells/mL) under hypoxic conditions [22] in the presence and absence of 3 mM KCN. The cell suspension, KCN and drug stock solutions were deoxygenated separately, and the KCN was added to the cells 5 min before the addition of **7**. The concentration of parent drug and its amine reduction product in the extracellular medium was determined at various times by centrifuging samples to pellet cells, and injecting the supernatant (200 μL) directly into the HPLC system. The chromatographic method was as described previously [21]. The amine metabolite was identified by comparison of retention time and absorbance spectrum with the synthetic 5-amine [21].

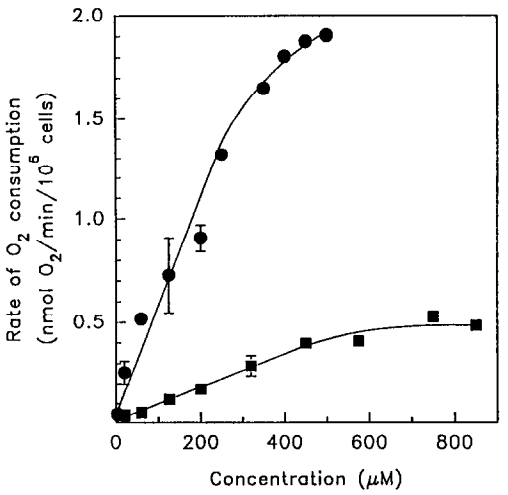


Fig. 2. Rate of drug-induced O_2 consumption in cyanide-inhibited AA8 cells as a function of concentration for (●) compound **7**, and (■) compound **11**. Where error bars are shown, values are means ± SEM, N = 3; other points are single determinations.

Statistical analysis. Linear regression was performed using SigmaStat Version 1 statistical software (Jandel Scientific) and multiple linear regression using SAS/STAT Version 6 (SAS Institute Inc.).

RESULTS AND DISCUSSION

Drug concentration dependence of oxygen consumption. The rates of O_2 consumption induced by the 8-methyl 5-nitroquinoline derivative (**7**) and by its 3,8-dimethyl substituted analogue (**11**) showed an approximately linear concentration dependence at ≤ 400 μM (Fig. 2). At higher drug concentrations, the rate of O_2 consumption did not increase linearly, possibly reflecting saturation of uptake or metabolism, with apparent K_m values (estimated from double-reciprocal plots) of *ca.* 1.2 mM for both compounds. A drug concentration of 200 μM was used to compare rates of O_2 consumption induced by other analogues.

E_7^1 dependence of drug-induced oxygen consumption. Rates of O_2 consumption were compared for an extended series of 4-alkylaminonitroquinolines, which included all the synthetically accessible nitro regioisomers (with the nitro groups in the 3-, 5-, 6-, 7- and 8-positions), 5-NO_2 derivatives with substituents on the quinoline ring to modify reduction potential, and one analogue (**3**) with a neutral hydrophilic 4-substituent in place of the basic dimethylaminopropylamino side chain. The com-

* Atwell GJ, Wilson WR, Anderson RF and Denny WA, Manuscript in preparation.

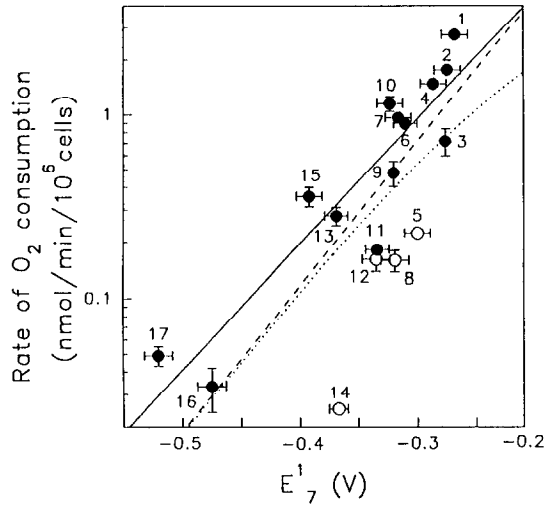


Fig. 3. Rates of O_2 consumption induced by 200 μM 4-alkylaminonitroquinolines in cyanide-inhibited AA8 cells. Key: (○) 6-substituted 5-nitro compounds (*ortho* substituents), and (●) all other compounds. Compound **14** did not stimulate oxygen consumption significantly, and is plotted at the sensitivity limit of the assay (0.025 nmol O_2/min/10^6 cells). Error bars are SEM (data from Table 1). The solid line is the first-order regression through the filled symbols. Predicted values of R based on the scheme shown in Fig. 1 (see text) are shown, assuming $k_4 = 0$ (dashed line) or $k_4 = 50\,sec^{-1}$ (dotted line).

pounds are listed in Table 1 in rank order of E_7^1 values, which covered a range from −268 to −520 mV although only two examples (**16** and **17**) were below −400 mV.

O_2 consumption induced by the nitroquinolines at 200 μM (Table 1) showed an obvious positive correlation with E_7^1 (Fig. 3). The dependence of the rate of drug-stimulated O_2 consumption (R in units of nmol/min/10^6 cells) on E_7^1 (in volts) was assessed by linear regression of Equation 1:

$$\log R = aE_7^1 + b. \quad (1)$$

There was a statistically significant linear correlation ($r = 0.819$, $F = 28.2$, $P < 0.001$) between log R and E_7^1 for the set of all nitroquinolines, excluding the 3,6-dimethyl compound (**14**), which did not detectably stimulate oxygen consumption. The coefficients for this correlation were slope a = 6.4 V^{-1} and intercept b = 1.81.

Inspection of the data (Fig. 3) indicated that not only **14** but all four 5-nitroquinolines with a substituent in the 6-position appeared to induce lower rates of oxygen consumption than expected from their E_7^1 values. Introducing a binary variable (I) for 6-substitution into the regression analysis (Equation 2; I = 0 for 6-substituted compounds, I = 1 for 6-unsubstituted compounds) gave a significant improvement in the correlation [F statistic for the regression increased from 20.7 to 43.4; further, the F statistic for the introduction of the binary variable (28.3) was highly significant (P =0.0001)].

$$\log R = 7.1E_7^1 + 0.74I + 1.39. \quad (2)$$

The coefficient for I (0.74) indicates that the presence of a small substituent (Me, Et) in the 6 position decreases R by *ca.* 5-fold. Boyd *et al.* [29] demonstrated by O^{17} NMR that substitution *ortho* to the nitro group in the 4-alkylaminonitroquinolines provides severe steric compression, resulting in twisting of the nitro group so that in compound **8** it is approximately orthogonal to the plane of the quinoline chromophore. The present study indicates that, in addition to any effect which these steric interactions might have on E_7^1, there is a steric effect of *ortho* substitution on the net rate of redox cycling in cells. This could, in principle, be due to effects on any one of three processes: cellular uptake, enzymatic reduction of the nitro compound, or reactivity of the nitro radical anion with O_2. However, studies with **14** show that the intracellular concentration of this compound in aerobic AA8 cells is similar to that for compounds that induce more rapid O_2 consumption (**4**, **7**, **11** and **13**) [30], indicating that the lack of redox cycling is not due to poor cellular uptake. The net reduction of (**14**) to its corresponding amine in hypoxic AA8 cells has also been measured [21], and is *ca.* 15-fold slower than for the corresponding 3,8-dimethyl isomer **11**, which has a similar E_7^1. The latter study, therefore, provides evidence, independent of the present demonstration of *ortho* effects on oxygen consumption, for steric inhibition by 6-alkyl substitution of reduction at the active site of the nitroreductase(s) responsible for one-electron reduction in this series of 5-nitroquinolines.

The existence of specific substituent effects on cellular reduction, despite the relatively broad collective specificity of the various nitroreductases in cells, points to the importance of direct measurement of rates of nitroreduction rather than the use of E_7^1 values as a surrogate measure of these rates. It also demonstrates the possibility of controlling reduction rates independently of reduction potential. Such steric manipulation of enzymatic reduction rates may be of particular advantage in the further development of nitro compounds as hypoxic cell radiosensitizers; if the reaction with DNA radicals is less sterically demanding than interaction with the active sites of nitroreductases, then inhibition of enzymatic activation (and hence toxicity) may be achieved without compromising radiosensitizing potency.

The above dependence of log R on E_7^1 (slope 7.1 V^{-1}, Equation 2) was slightly less than the value of 10 predicted by the Marcus theory for a one-electron transfer process [31]. Three other studies have investigated the relationship between reduction potential and rates of O_2 consumption in cyanide-inhibited cells. Biaglow *et al.* [14] demonstrated stimulation of O_2 consumption by 7 nitrobenzenes in Ehrlich ascites cells; these data do not indicate a statistically significant correlation of either R or log R with $E_{1/2}$ for this small set of compounds, although a correlation (slope of log R vs $E_{1/2}$ *ca.* 3 V^{-1} was observed in a subsequent study with a set of 18 nitroheterocycles using microsomes from these cells [32]. Analysis of the data of Zeman *et al.* [33] on O_2

consumption stimulated by benzotriazine di-*N*-oxides in CHO cells indicates a significant (r = 0.872, F = 48, P < 0.001, N = 16) linear correlation between log R and $E_{1/2}$, with a slope of 10.4 V^{-1}. In contrast, a slope of only 3.3 V^{-1} was obtained by linear regression of log R and E_7^1 for nitrobenzenes in hepatocytes (r = 0.970, F = 94, P < 0.001, N = 12), using data reported by O'Brien *et al.* [34]. The reasons for the differences between these studies are not clear, but may reflect differences in the enzymology of reduction with different substrates or cell types.

A complication in interpreting the above studies is the lack of information on K_m values. In the present study, the chosen substrate concentration (200 μM) was below the apparent K_m value (1.2 mM) for compounds **7** and **11**. If saturation of cellular uptake contributes to the apparent K_m, the K_m values for enzymatic nitroreduction will be even higher. Under these conditions the pseudo first-order rate constant for reduction approximates to V_{max}/K_m. Orna and Mason [35] have demonstrated a correlation between log (V_{max}/K_m) and E_7^1 for O_2 consumption by aerobic nitroreduction using purified flavo-enzymes, with a slope of 13.5 to 15 V^{-1}. However, if the only redox-dependent step is electron transfer in the enzyme–substrate complex, then the E_7^1 dependence should be dictated by that for V_{max} alone and, as noted by the above authors, the apparent involvement of K_m in the redox dependence is unexplained. In contrast, the Marcus parameters for enzymatic (xanthine oxidase) and non-enzymatic ($FMNH_2$) reduction of alkylaminonitroacridines, closely related to the present series of nitroquinolines, indicated a stronger correlation of E_7^1 with V_{max} than with V_{max}/K_m [36]. Thus, the observed relationship between E_7^1 and log R in the present study suggests that the K_m values of all the alkyl-aminonitroquinolines are broadly similar with the exception of the 6-substituted 5-nitro derivatives, which are inferred to have higher K_m values as a result of steric interference with binding to the active site. Unrecognized variation in K_m values in other studies with intact cells may have partially obscured the redox dependence of oxygen consumption.

Kinetic modelling of rates of drug-induced O_2 *consumption in respiration-inhibited cells.* In the nitroquinoline series, there is sufficient information on the rates of the component reactions of Fig. 1 to ask whether the observed rates of O_2 consumption (and the apparently linear relationship between log R and E_7^1) are consistent with this kinetic model. This analysis draws on two sources of information. (i) The rate of loss of the 4-alkylaminonitroquinolines in hypoxic AA8 cultures, as determined by Siim *et al.* [21], provides the rate constants for the initial one-electron reduction (k_1), and its dependence on E_7^1, since the initial one-electron reduction is rate-limiting in this system [21]. This approach makes the key assumption that k_1 is the same in aerobic and anoxic cells. It also requires that KCN does not inhibit nitroreduction under the conditions of the O_2 consumption experiments. The latter was tested for compound **7** by measuring rates of net reduction under hypoxia; nitro compound loss and amine formation was not appreciably inhibited by 3 mM KCN (Fig. 4). (ii) The rate constants for the reaction of the nitro radicals with O_2 (k_2), and for their disproportionation (k_3), can be estimated from the known reaction kinetics for these and other nitro radicals, as determined by pulse radiolysis in dilute solution.

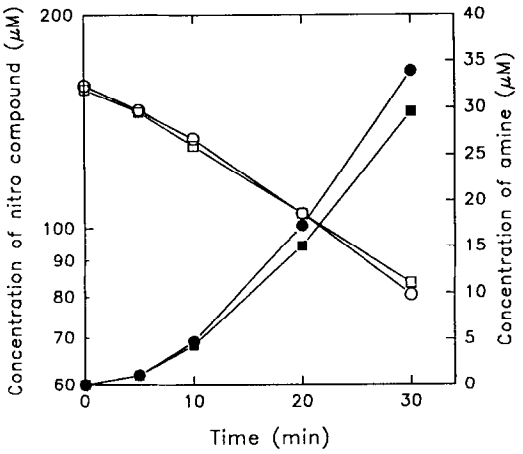


Fig. 4. Reduction of compound **7** by hypoxic AA8 cells (10^7/mL) as determined by HPLC of extracellular medium. Open symbols: parent nitro compound; filled symbols: corresponding amine. Circles: minus KCN; squares: plus 3 mM KCN. Values are for a single experiment.

Regression of the first-order rate constants, k_{obs} (in units of sec^{-1}), for net nitro reduction under hypoxia at 10^6 cells/mL (determined from the first half-life for drug loss) against E_7^1 (in volts), using the published data for compounds **4**, **7**, **11**, **13** and **17** [21], provides:

$$\log k_{obs} = 7.9E_7^1 - 1.86. \quad (3)$$

Under anoxia, for every 2 moles of $ArNO_2^{\cdot-}$ produced, one mole of $ArNO_2$ is regenerated by disproportionation (assuming the rates of any other reactions of $ArNO_2^{\cdot-}$ are negligible) and therefore $k_1 = 2k_{obs}$. Substituting in Equation 3 gives:

$$\log k_1 = 7.9E_7^1 - 1.56. \quad (4)$$

The value of the second-order rate constant k_2 for each drug was calculated from the dependence of k_2 on E_7^1 reported by Wardman and Clarke [8], who used pulse radiolysis to investigate this reaction for a wide variety of monocyclic nitro(hetero)arenes. Their data give:

$$\log k_2 = 4.05 - 6.0E_7^1 \quad (5)$$

where k_2 is in units of $M^{-1}sec^{-1}$ and E_7^1 is in volts. Equation 5 predicts k_2 for the reaction of the nitro radical anion of **7** with O_2 to be 8.8×10^5 $M^{-1}sec^{-1}$, in good agreement with the experimentally determined value of 8×10^5 $M^{-1}sec^{-1}$ at pH 7.0**.

The second-order rate constant k_3 for the disproportionation of $ArNO_2^{\cdot-}$ is generally considered to be independent of the reduction potential

** P. Wardman, personal communication. Cited with permission.

of the parent compound, but is dependent on pH and radical pK_a [12]. A constant value for k_3 ($2.5 \times 10^6\,M^{-1}sec^{-1}$, which is half the value of k_{obs} for second-order decay of 7 at pH 7**) has been assumed for the present series. The rate constant k_4 for competing first-order decay of $ArNO_2^{\cdot-}$ was also assumed to be equal for all compounds, and values from 0 to $50\,sec^{-1}$ were examined (k_4 is 5–$10\,sec^{-1}$ for most simple 2-nitroimidazoles in the absence of biological molecules, and is less than this for 5-nitroimidazoles [3, 12]). The model is not sensitive to the value assumed for the rate constant for superoxide dismutation (k_5) provided that the rate is sufficient that $O_2^{\cdot-}$ is in steady state over the measurement period. This requirement is readily met by the rate constant of $2 \times 10^5\,M^{-1}sec^{-1}$ for spontaneous disproportionation of $O_2^{\cdot-}$ at pH 7.4 [37] even if cyanide completely inhibits superoxide dismutase [38].

The predicted value of $R(-dO_2/dt)$ is given by:

$$R = k_2[O_2][ArNO_2^{\cdot-}] - k_5[O_2^{\cdot-}]^2. \quad (6)$$

Solving for $[ArNO_2^{\cdot-}]$ and $[O_2^{\cdot-}]$ under steady-state conditions and substituting into Equation 6 yields:

$$R = k_2[O_2]\{-(k_2[O_2] + k_4 + ((k_2[O_2] + k_4)^2 + 8k_1k_3[ArNO_2])^{\frac{1}{2}}\}/8k_3. \quad (7)$$

Values of R were calculated from Equation 7, using estimates of k_1 and k_2 from Equations 4 and 5, and with initial concentrations of $ArNO_2^{\cdot-}$ and O_2 both equal to 200 μM. These predicted rates are plotted as a function of E_7^1 and compared with the experimentally determined values in Fig. 3. The predicted values are in good agreement with observation (excluding the 6-substituted 5-nitro compounds) assuming $k_4 = 0$, although they are slightly lower than the measured values. The predicted rates show little dependence on k_4 up to values of $50\,sec^{-1}$, and fit the experimental data best when $k_4 = 0$. This suggests that there are no significant routes of consumption of $ArNO_2^{\cdot-}$ in cells other than disproportionation and transfer of the electron to O_2. Predicted values of R were also insensitive to the value used for k_3 provided this was $< 10^7\,M^{-1}sec^{-1}$, indicating that at the very low steady-state concentration of the nitro radical anion in cells (calculated as 1.6×10^{-10} M for a nitroquinoline with an E_7^1 of -0.3 V, assuming $k_4 = 0$) the kinetically favoured reaction is oxidation by O_2 rather than disproportionation. This is consistent with the essentially complete inhibition of net nitro reduction (loss of nitro compound and amine formation) of 4-alkylamino-5-nitroquinolines in aerobic AA8 cultures [21]. Orna and Mason [35] have similarly concluded, on the basis of studies with purified flavoenzymes, that oxygen competes successfully with net reduction via disproportionation if the steady-state radical concentration is in the order of 10^{-8} M or less.

The predicted dependence of log R on E_7^1 over this range of reduction potentials (< -0.2 V) was approximately linear, when $k_4 = 0$, with a slope of $7.9\,V^{-1}$. This is in accord with expectation since the limiting slope at low reduction potential will be determined by the dependence of k_1 on E_7^1 (Equation 4). The expected slowing of rates of oxygen consumption at high reduction potential (due to the decrease in k_2) is only significant at E_7^1 values well above the range examined experimentally. (At $E_7^1 = 0$ V, the value of R estimated by the model is only 5% below the linear extrapolation of the dashed line in Fig. 3.)

The success of this simple kinetic model in predicting oxygen consumption rates supports the assumptions implicit in the above analysis. An important assumption is that k_1 is the same under aerobic and anoxic conditions, i.e. that inhibition by O_2 is only a consequence of its back-oxidation of the nitro radical, not a decrease in the rate of forward reduction as might occur if the supply of reducing equivalents were greater in hypoxic cells. Any lowering of k_1 under aerobic conditions would further lower the predicted values and thus cannot account for the difference between observed and predicted values. Similarly, any increase in expression of nitroreductases under hypoxic conditions [39] would lower the predicted oxygen consumption values and thus worsen the discrepancy.

The above kinetic analysis implicitly assumes homogeneous chemical kinetics (the effective rate constants are averaged over the entire culture volume), although the nitro radical will be generated only within cells. It appears that nitro radicals do diffuse from cells readily [11], but if it is assumed that the nitro radical anion is confined to the cell, then the effective intracellular value of k_1 should be increased by *ca.* 1000-fold relative to that estimated at 10^6 cells/mL from Equation 4 by averaging over the entire culture volume. Under these conditions, Equation 7 predicts that the competition between radical oxidation and disproportionation (net reduction) will shift in the direction of the latter. However, this effect is very small over the range of reduction potentials examined; for a nitroquinoline with a reduction potential of -0.3 V, the volume-averaged O_2 consumption rate at high O_2 would be only 0.006% less if the radical is confined to the cell than if it were distributed homogeneously (assuming that the intracellular and extracellular O_2 concentrations are the same, which is a reasonable approximation at these high oxygen concentrations). Thus, the above kinetic treatment is insensitive to the assumption of isotropic radical distribution.

In conclusion, a simple homogeneous kinetic model can quantitatively predict the measured rates of one-electron redox cycling for this series of 4-alkylaminonitroquinolines in AA8 cells. The model accounts for the observed dependence of O_2 consumption on E_7^1. This suggests that the key assumptions are valid, namely that the rate of the forward one-electron reduction reaction is independent of O_2, that there are no major fates of nitro radical anions in cells other than reaction with other nitro radicals or O_2, and that the radical reaction kinetics observed in dilute solution are directly applicable in intact cells. Redox cycling of 4-alkylaminonitroquinolines clearly operates at close

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to 100% efficiency in aerobic cells. Therefore, these compounds are useful model oxidants for the investigation of redox cycling, and provide the basis for recent quantitative investigations [22] of the oxygen dependence of activation of nitroaromatic bioreductive drugs.

Acknowledgements—We thank Prof. William A. Denny and Graham J. Atwell for synthesis of the nitroquinoline derivatives, Drs. Frederik B. Pruijn and Robert F. Anderson for helpful discussions, and Mrs. Dianne Ferry for technical assistance. This study was funded by the Health Research Council of New Zealand. B.G.S. was the recipient of a Postgraduate Scholarship from the University Grants Committee of New Zealand.

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