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

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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) 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, suggesting that interaction with the active site of nitroreductases is impeded sterically for such compounds. Absolute values of R (and the observed E 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⁻) as the first intermediate in the reduction pathway [2]. Further reduction is considered to proceed by disproportionation of ArNO⁻, in competition with its reoxidation by O₂ [2-4] (Fig. 1). The reaction of ArNO⁻ with O₂ is responsible for generation of O₂⁻, and hence toxicity due to reactive oxygen species [5, 6]. It is also responsible for the inhibition of net nitroreduction by O₂ [7, 8]; the further reduction to reactive nitroso and hydroxylamine derivatives in the absence of O₂ 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 O₂ and generation of H₂O₂ 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 O₂ consumption induced by nitro compounds in whole cells. In particular, it is unclear

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Fig. 1. Scheme for the one-electron reduction of nitro compounds and the operation of a futile redox cycle in the presence of oxygen. k₁ (etc.) are the rate constants for the indicated reactions.
Table 1. Drug structures, E\textsubscript{1} values and rates of drug-induced oxygen consumption in cyanide-inhibited AA8 cells by 4-alkylaminonitroquinolines at 200 \mu M

| No. | Structure formula; R = E\textsubscript{1}* (mV)\textsuperscript{t} Rate of drug-induced oxygen consumptiont (nmol/min/10\textsuperscript{6} cells) |
|-----|-------------------------------------------------|-------------------------------------------------|
| 1   | A; 8-NO\textsubscript{2}                         | -268 ± 11\textdagger+ 2.739 ± 0.022            |
| 2   | A; 2-Me 5-NO\textsubscript{2}                   | -274 ± 11\textdagger+ 1.754 ± 0.070            |
| 3   | B                                               | -276$                                  0.713 ± 0.121 |
| 4   | A; 5-NO\textsubscript{2}                       | -268 ± 11\textdagger+ 1.470 ± 0.037\textdagger+ |
| 5   | A; 6-Et 5-NO\textsubscript{2}                  | -300 ± 11\textdagger+ 0.223 ± 0.008           |
| 6   | A; 8-OMe 5-NO\textsubscript{2}                 | -310 ± 1\textdagger+ 0.889 ± 0.060            |
| 7   | A; 6-NO\textsubscript{2}                       | -316 ± 1\textdagger+ 0.960 ± 0.067           |
| 8   | A; 6-Me 5-NO\textsubscript{2}                  | -319 ± 12$                              0.159 ± 0.021 |
| 9   | A; 2,3-diMe 5-NO\textsubscript{2}              | -320$                      0.477 ± 0.074           |
| 10  | A; 7-NO\textsubscript{2}                       | -323 ± 11\textdagger+ 1.145 ± 0.107           |
| 11  | A; 3,8 diMe 5 NO\textsubscript{2}              | -334 ± 10$                              0.182 ± 0.012\textdagger+ |
| 12  | A; 6,8-diMe 5-NO\textsubscript{2}              | -335 ± 12$                              0.161 ± 0.022           |
| 13  | A; 3-Me 5-NO\textsubscript{2}                  | -369 ± 10\textdagger+ 0.277 ± 0.032           |
| 14  | A; 3,6-diMe 5-NO\textsubscript{2}              | -367 ± 8$                              <0.025            |
| 15  | A; 6-NO\textsubscript{2}                       | -392 ± 11\textdagger+ 0.355 ± 0.044           |
| 16  | A; 3-NO\textsubscript{2}                       | -475 ± 12$                             0.033 ± 0.009           |
| 17  | A; 8-NHMe 5-NO\textsubscript{2}                | -570 ± 17$                              0.049 ± 0.006\textdagger+ |

* Determined by pulse radiolysis in aqueous solutions containing 0.2 M propan-2-01, 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\textsubscript{1} for the redox indicator benzyl viologen to be -374 mV [20].
$ P. Wardman, personal communication. Cited with permission.
|| Data from Ref. 21.
\textdagger+ R. F. Anderson, personal communication. Cited with permission.

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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} consumption in cyanide-inhibited AA8 cells.
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$^+$ and demonstrated that there is a specific substituent effect on nitroreduction that is not mediated via changes in E$^+$. 

**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°C.

**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 (~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$^6$/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°C 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°C, 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 (1 mL at 10$^6$ 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].

**Statistical analysis.** Linear regression was performed using SigmaStat Version 3 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$^+$ 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-

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The coefficients for this correlation were \( a = -5.20 \) from their \( E^+ \) values. Introducing a binary variable \( \log R \) and \( E^+ \) for the set of all nitroquinolines, values, which did not detectably stimulate oxygen consumption. 

6.4 V and intercept \( b = 1.81 \). 

The above dependence of \( \log R \) on \( E^+ \) (slope 7.1 V, 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 \( E^+ \) for this small set of compounds, although a correlation (slope of \( \log R \) vs \( E^+ \), ca. 3 V) 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 \( O_2 \) 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 \( E^+ \) 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 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 \( O^{13} \) NMR that substitution \( \text{ortho} \) to the nitro group in the 4-alkylaminonitroquinolines provides severe steric compression, resulting in twisting of the nitro group 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 \( E^+ \), there is a steric effect of \( \text{ortho} \) 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 14 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 \( E^+ \). The latter study, therefore, provides evidence, independent of the present demonstration of \( \text{ortho} \) 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.

**Fig. 3.** Rates of \( O_2 \) consumption induced by 200 M 4-alkylaminonitroquinolines in cyanide-inhibited AA8 cells. Key: (C) 6-substituted 5-nitro compounds (\( \text{ortho} \) 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 \( k_a = 0 \) (dashed line) or \( k_a = 50 \text{ sec}^{-1} \) (dotted line). 

Predicted values of \( R \) based on the scheme shown in Fig. 1 (see text) are shown, assuming \( k_a = 0 \) (dashed line) or \( k_a = 50 \text{ sec}^{-1} \) (dotted line)
Redox cycling of nitroquinoline bioreductive drugs

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 $E_i$ 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 $E_i$ 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 $\mu$M) 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 $E_i$ for $O_2$ consumption by aerobic nitroreduction using purified flavoenzymes, 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 $E_i$ 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 $E_i$ with $V_{max}$ than with $V_{max}/K_m$ [36]. Thus, the observed relationship between $E_i$ and log $R$ in the present study suggests that the $K_m$ values of all the alkylaminonitroquinolines 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 $E_i$) 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 Simm et al. [21], provides the rate constants for the initial one-electron reduction ($k_3$), and its dependence on $E_i$, since the initial one-electron reaction is rate-limiting in this system [21]. This approach makes the key assumption that $k_3$ 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.

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

$$\log k_{obs} = 7.9E_i - 1.86.$$

Under anaerobia, for every 2 moles of ArNO$_2$• produced, one mole of ArNO$_2$ is regenerated by disproportionation (assuming the rates of any other reactions of ArNO$_2$• are negligible) and therefore $k_2 = 2k_{obs}$. Substituting in Equation 3 gives:

$$\log k_2 = 7.9E_i - 1.56.$$

The value of the second-order rate constant $k_2$ for each drug was calculated from the dependence of $k_2$ on $E_i$ 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:

$$\log k_2 = 4.05 - 6.0E_i$$

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

The second-order rate constant $k_3$ for the disproportionation of ArNO$_2$• 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 pKα [12]. A constant value for k₃ (2.5 × 10⁶ M⁻¹sec⁻¹, which is half the value of kobs for second-order decay of 7 at pH 7***) has been assumed for the present series. The rate constant k₃ for competing first-order decay of ArNO⁻ is also assumed to be equal for all compounds, and values from 0 to 50 sec⁻¹ were examined (k₃ 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 (k₄) provided that the rate is sufficient that O₂⁻ is in steady state over the measurement period. This requirement is readily met by the rate constant of 2 × 10⁶ M⁻¹sec⁻¹ for spontaneous disproportionation of O₂⁻ at pH 7.4 [37] even if cyanide completely inhibits superoxide dismutase [38].

The predicted value of R(-dO₂/dt) is given by:

\[ R = k₃[O₂][ArNO₂⁻] - k₃[O₃⁻]². \]  

(6)

Solving for [ArNO₂⁻] and [O₃⁻] under steady-state conditions and substituting into Equation 6 yields:

\[ R = k₃[O₂][ArNO₂⁻] + k₄ + [(k₃[O₂] + k₄)² + 8k₃k₄[ArNO₂⁻]]/8k₃. \]  

(7)

Values of R were calculated from Equation 7, using estimates of k₃ and k₄ from Equations 4 and 5, and with initial concentrations of ArNO₂⁻ and O₂ both equal to 200 μM. These predicted rates are plotted as a function of E⁺ 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 k₄ = 0, although they are slightly lower than the measured values. The predicted rates show little dependence on k₃ up to values of 50 sec⁻¹, and fit the experimental data best when k₃ = 0. This suggests that there are no significant routes of consumption of ArNO₂⁻ in cells other than disproportionation and transfer of the electron to O₂. Predicted values of R were also insensitive to the value used for k₃ provided this was <10⁶ M⁻¹sec⁻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⁺ of -0.3 V, assuming k₄ = 0) the kinetically favoured reaction is oxidation by O₂ 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 10⁻¹⁸ M or less.

The predicted dependence of log R on E⁺ over this range of reduction potentials (< -0.2 V) was approximately linear, when k₄ = 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 k₄ on E⁺ (Equation 4). The expected slowing of rates of oxygen consumption at high reduction potential (due to the decrease in k₄) is only significant at E⁺ values well above the range examined experimentally. (At E⁺ = 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 k₃ is the same under aerobic and anoxic conditions, i.e. that inhibition by O₂ 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 k₄ 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 k₄ should be increased by ca. 1000-fold relative to that estimated at 10⁷ 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 O₂ consumption rate at high O₂ 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 O₂ 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 aerobic AA8 cultures [21]. The model accounts for the observed dependence of O₂ consumption on E⁺. This suggests that the key assumptions are valid, namely that the rate of the forward one-electron reduction reaction is independent of O₂, that there are no major fates of nitro radical anions in cells other than reaction with other nitro radicals or O₂, 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
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.

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