Free Rad. Res., Vol. 25, No. 2, pp. 99–107
Reprints available directly from the publisher
Photocopying permitted by license only

Electron Transfer Reactions in RB90745, A Bioreductive Drug Having Both Aromatic *N*-Oxide and Nitroarene Moieties

K. INDIRA PRIYADARSINI[1,3], MATTHEW A. NAYLOR[2], MICHAEL R. L. STRATFORD[1] and PETER WARDMAN[1]*

[1]*Gray Laboratory, P O Box 100, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, UK,* [2]*Department of Medicinal Chemistry, MRC Radiobiology Unit, Chilton, Didcot, Oxon, OX11 ORD, UK,* [3] *Chemistry Division, Bhabha Atomic Research Centre, Bombay-400085, India*

Accepted by Professor H. Sies

(Received November 20th, 1995; in final form January 22nd, 1996)

The bifunctional hypoxia-specific cytotoxin RB90745, has a nitroimidazole moiety attached to an imidazo[1,2,-*a*]quinoxaline mono-*N*-oxide with a spacer/linking group. The reduction chemistry of the drug was studied by pulse radiolysis using the one electron reductant $CO_2^{\bullet-}$. As N-oxides and nitro compounds react with $CO_2^{\bullet-}$ at diffusion controlled rates, initial reaction produced a mixture of the nitro radical (λ_{max} 410 nm) and the *N*-oxide radical (λ_{max} 550 nm) in a few microseconds. Subsequently an intramolecular electron transfer (IET) was observed ($k = 1.0 \pm 0.25 \times 10^3$ s^{-1} at pH 5–9), from the *N*-oxide to the more electron-affinic nitro group. This was confirmed by the first order decay rate of the radical at 550 nm and formation at 410 nm, which was independent of both the concentration of the parent compound and the radicals. The rates of electron transfer and the decay kinetics of the nitro anion radicals were pH dependent and three different pK_as could be estimated for the one electron reduced species: 5.6 (nitroimidazole group) and 4.3, and 7.6 (*N*-oxide function). The radicals react with oxygen with rate constants of 3.1×10^7 and 2.8×10^6 dm^3 mol^{-1} s^{-1} observed at 575 nm and 410 nm respectively. Steady state radiolysis studies indicated four electron stoichiometry for the reduction of the compound.

Key words: bifunctional bioreductive drug, mono *N*-oxide, nitroimidazole, electron transfer

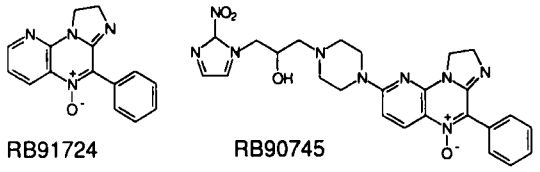
Abbreviations: IET, intramolecular electron transfer

INTRODUCTION

Aromatic compounds with di and mono *N*-oxide functions like 1,2,4-benzotriazine-1,4-di-oxides and imidazo[1,2-*a*]quinoxaline mono *N*-oxides have been shown to have selective toxicity for hypoxic cells.[1–8] The hypoxic selective toxicity of these compounds arises mainly from the *N*-oxide function, via the one electron reduced intermediates, where the *N*-oxide is reduced by the reductase and in well oxygenated cells the drug is restored by electron transfer to oxygen.

Current interest is also focused on dual-function compounds which represent a new classes of bioreductives. An earlier example of a

*Correspondence Address: Prof. P. Wardman, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, UK. Fax: 44-1923-835210



compound of this type is RSU-1069 which contains a weakly basic alkylating agent attached to the nitro group.[9,10] It has been shown that the dual function derivatives are more effective in causing DNA damage under hypoxia than the monofunctional drugs. A new bifunctional bioreductive RB90745 was synthesised by Naylor *et al.* that has both imidazo[1,2-a]quinoxaline mono *N*-oxide and a nitroarene function linked through a spacer.[4] The compound contains two different types of bioreductive moieties with different mechanisms of cytotoxicity. We investigated the reduction chemistry and electron transfer reactions of RB90745 with a view to understanding the effect of introducing a nitro group on the bioreductive *N*-oxide and compared the chemical properties of the radicals with the unsubstituted mono *N*-oxide RB91724.

EXPERIMENTAL

Materials

RB90745 and RB91724 were synthesised according to the methods described.[4] Sodium formate, potassium thiocyanate and phosphate salts (analytical grade) were obtained from Merck. All solutions were prepared in water from a 'Milli-Q' system (Millipore). Phosphate salts (5 mmol dm^{-3}) were used in varying proportion to adjust the pH in addition to NaOH and $HClO_4$ wherever required. Gases N_2O, O_2 and N_2O-O_2 were from British Oxygen Co.

Methods

The pulse radiolysis experiments were performed with a 4 MeV Van de Graaff accelerator; 30 ns electron pulses with typical absorbed doses of 2–3 Gy were used for most of the studies except to determine the second order rate constants where the absorbed doses were increased up to 15–20 Gy. The pulse radiolysis methodology with absorption detection at the Gray Laboratory has been previously described.[11] The absorbed dose was determined using thiocyanate dosimetry monitoring $(SCN)_2^{\bullet-}$ at 472 nm. Pulse radiolysis studies were carried out in N_2O-saturated aqueous solutions containing 0.1 mol dm^{-3} sodium formate and phosphate buffer utilizing a model one-electron reductant $CO_2^{\bullet-}$ as a mimic for reductase enzyme activity in cells.

Radiolysis of water produces three highly reactive species $H^\bullet$, $^\bullet OH$ and e^-_{aq}, besides less reactive molecular species. In N_2O saturated solutions e^-_{aq} is quantitatively converted to $^\bullet OH$ ($N_2O + e^-_{aq} \rightarrow {}^\bullet OH + OH^- + N_2$)[12]. Both $^\bullet H$/$^\bullet OH$ were converted to one-electron reducing $CO_2^{\bullet-}$ ($CO_2/CO_2^{\bullet-}$ −1.8 V vs. NHE), using sodium formate ($^\bullet H/{}^\bullet OH + HCO_2^- \rightarrow CO_2^{\bullet-} + H_2O/H_2$). Thus in the presence of 0.1 mol dm^{-3} sodium formate and N_2O, the radiation chemical yield of $CO_2^{\bullet-}$ is 0.68 μmol J^{-1}[13].

Steady state radiolysis experiments of the

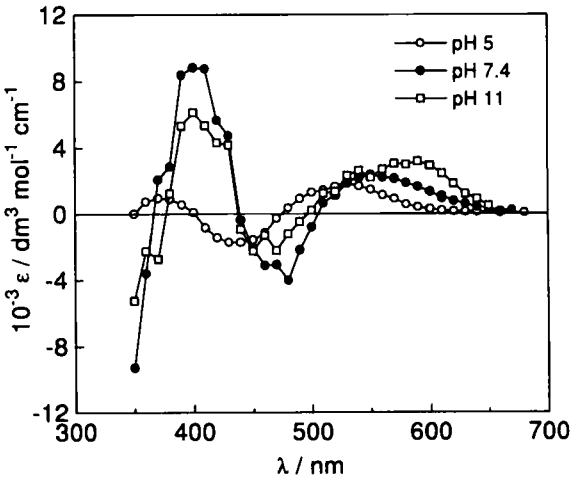


FIGURE 1 Differential absorption spectrum (radical minus ground state) of the radical anions of RB90745, 40–60 μs after the pulse at pH 5 (o), 7.4 (●) and 11 (□), generated on pulse radiolysis of N_2O saturated solutions of RB90745 (50 μmol dm^{-3}) and formate (0.1 mol dm^{-3}).

compounds were carried out in 50 ml syringes using a Co^{60} γ-source, with a dose rate of 6.99 Gy min^{-1} as determined by Fricke dosimetry. The solutions were saturated with N_2O prior to irradiation. Analysis of compounds by High Performance Liquid Chromatography (HPLC) utilised a base-deactivated reversed-phase column (Hichrom RPB, 100 mm × 4.6 mm), using linear gradients of phosphate buffers and acetonitrile. The flow rate was 2 ml/min. Detection was by absorbance at 254 nm using a variable wavelength detector (Waters 486). The ion pairing agent heptane sulphonic acid as the sodium salt was introduced in order to adequately resolve the radiolysis products.

RESULTS

Absorption Spectra of the One-electron Reduced Radicals

The transient species formed by one-electron reduction of the compound RB90745, by $CO_2^{\bullet-}$ were studied (~50 μmol dm^{-3} drug, 0.1 mol dm^{-3} formate, N_2O, 5 mmol dm^{-3} phosphate). The transient exhibits absorption in the wavelength range ~300–700 nm with two distinct absorption bands: ~400 nm and another broad band at 550–600 nm. Its formation was complete in a few microseconds, from which rate constants for the one electron reduction by $CO_2^{\bullet-}$ were determined to be $(2 \pm 1) \times 10^9$ and $(1.8 \pm 0.4) \times 10^9$ dm^3 mol^{-1} s^{-1} as measured by the radical absorption at 400 nm and 550 nm respectively. The absorption spectra of the one-electron reduced radicals of RB90745 were determined at pH 5, 7.4 and 11 after the reaction with the formate radicals was complete i.e. after 50–60 μs (Figure 1). The negative absorption at 400–480 nm is indicative of bleaching of the parent at these wavelengths, which changed with pH according to the pK_a of the parent. At pH 7.4 and 11 the absorption at 400 nm is stronger than the 550–600 nm absorption, whereas at pH 5, the absorption at 400 nm is shifted to 360–370 nm.

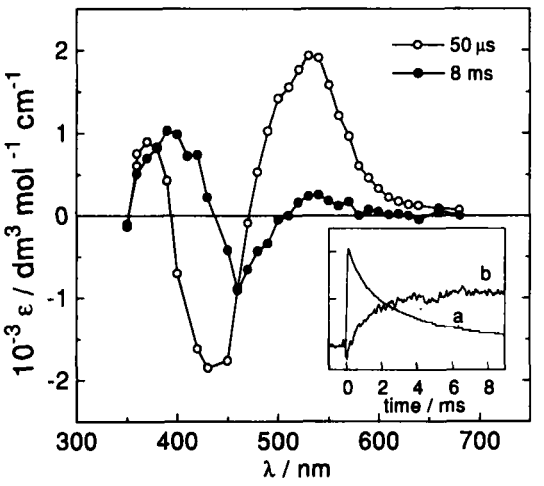


FIGURE 2 Time resolved absorption spectra of the radical anion of RB90745 (o) 50–60 μs and (●) 8 ms after the pulse, measured by the pulse radiolysis of N_2O saturated solutions of RB90745 (50 μmol dm^{-3}) and formate at pH 5. Inset: Absorption-time plots indicating (a) decay of the transient at 550 nm and (b) formation at 410 nm.

The time-resolved absorption spectra of the radical after 50–60 μs and 8 ms at pH 5 showed significant differences (Figure 2). After 8 ms, the absorption in the 550 nm region decayed almost completely, whereas in the 400 nm region, it increased. Similar time dependent spectral changes were observed at all the pHs. The transient absorption-time plots (inset of Figure 2) at these two wavelengths (410 nm and 550 nm) at pH 5 indicated almost complete decay at 550 nm and a rise in the absorption at 410 nm. The time scales for the decay of absorption at 550 nm and formation at 410 nm matched very well, suggesting a radical transformation presumably due to intramolecular electron transfer (IET) from the *N*-oxide function to the nitro group.

Effect of pH and the Prototropic Equilibria

Figure 1 indicates a change in the absorption spectrum of the radicals of RB90745 with pH, suggesting involvement of the different prototropic forms of the radicals and a prototropic equilibrium is expected for the one electron

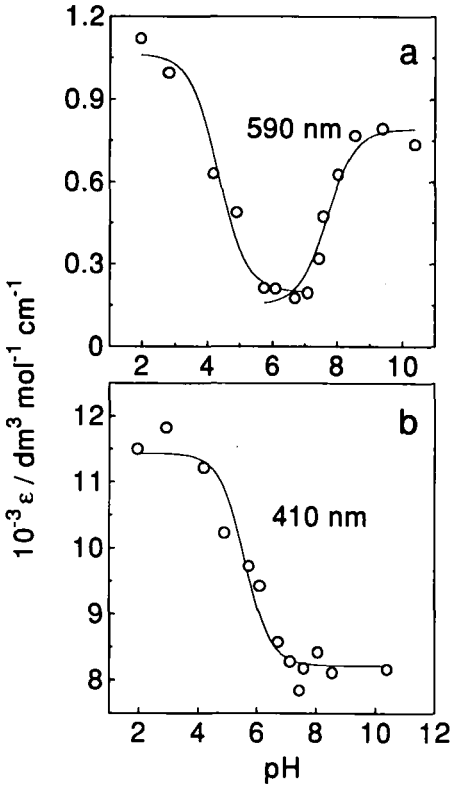


FIGURE 3 Effect of pH on the radical extinction coefficient (a) after 40–60 μs at 590 nm and (b) after a few ms at 410 nm (after complete formation). The radicals were generated by pulse radiolysis of N_2O saturated solutions of RB90745 (50 μmol dm^{-3}) and 0.1 mol dm^{-3} formate.

reduced radicals. The parent compound showed a pK_a of 6.2 ± 0.3 determined by following the absorption changes at 432 nm. As noted above, time resolved studies suggested formation of two different radicals (Figure 2). Since radical pK_a is a very useful parameter in assigning the nature of the transient, pK_a measurements were carried out at two different time scales corresponding to their absorption maxima i.e. at 590 nm: 40–60 μs after the pulse and at 410 nm: after a few ms. The sigmoidal curves representing changes in the extinction coefficients at 410 nm and 590 nm at these two different time scales, after correcting for the parent absorption are shown in Figures 3a and 3b. Fitting the curves allowed three pK_as to be estimated. The 410 nm absorption showed an inflection point at 5.6 ± 0.2, whereas the 590 nm absorption gave two inflection points at 4.3 ± 0.2 and 7.6 ± 0.1.

Kinetics of Electron Transfer and Radical Decay

The decay kinetics of the one-electron reduced radicals of RB90745 absorbing in the 550–600 nm region obeyed first-order, concentration-independent kinetics at low radical concentration (radiation dose). However at very high doses (>15 Gy), there was a small increase in the decay due to second-order radical-radical reactions. To verify whether it is a pseudo first order (dependent on the parent concentration) or a unimolecular process (independent of the parent concentration), we followed the effect of parent concentration on the decay kinetics. Changing the concentration of the parent from 25 μmol dm^{-3} to 100 μmol dm^{-3} affected neither the rate of decay at 550 nm nor the rate of formation at 410 nm, confirming a unimolecular process, i.e. an intramolecular transformation of the radical from the *N*-oxide to the nitro group.

In general the decay kinetics of the radicals of *N*-oxides and nitro compounds are very sensitive to the solution pH. For RB90745, the rates of decay of the radical at 550–600 nm and the formation at 410 nm were influenced by the pH. The first order rate observed at 550–600 nm region (data shown

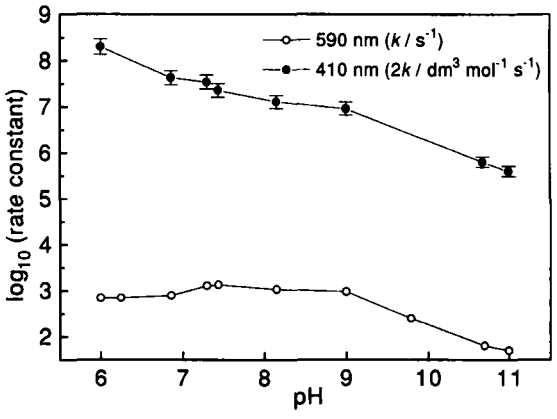


FIGURE 4 Rate constants for the decay of the radicals of RB90745 formed by reaction with $CO_2^{\cdot-}$ plotted on log scale over the pH range at 590 nm (o) (k, s^{-1}), and 410 nm (●) ($2k$, dm^3 mol^{-1} s^{-1}).

on log scale at 590 nm in Figure 4) increased from pH 7–9 and above pH 9 it decreased. However, the rate constant for the decay of the radical observed at 410 nm decreased significantly with pH ($2k$ on log scale in Figure 4). At pH >9 it has a lifetime of a few seconds ($2k \sim 4 \pm 0.4 \times 10^5$ dm^3 mol^{-1} s^{-1} at pH 11).

Reactivity with Oxygen

The reactivity of the radicals formed on one-electron reduction of RB90745 with oxygen was investigated by following the decay of the radicals at 575 nm and 410 nm in the presence of oxygen. For this purpose solutions at pH 9 were saturated with gaseous mixtures of nitrous oxide and oxygen in varying proportions from 1% to 10% oxygen (v/v). This corresponds to oxygen concentrations of 12.3 to 123 μmol dm^{-3} respectively. Low doses of radiation were used to generate only ~1–2 μmol dm^{-3} radicals so that radical-radical processes are slowed down. At high concentrations of oxygen only a fraction of $CO_2^{\bullet-}$ radicals reacted with the compound, reducing the yield of the drug radicals. The rate of decay of the radicals of RB90745 increased significantly in the presence of oxygen, observed at both 575 nm and 410 nm, and was first order in oxygen concentration. Rate constants were estimated from the slopes of the linear plots of the observed first order rate constants at 575 nm and 410 nm as a function of oxygen concentration (Figure 5). The effect of oxygen on the rate of decay is not the same at 575 nm and 410 nm (the former decayed faster than the latter: inset a and b of Figure 5) and the oxygen quenching rate constants were estimated to be $3.1 \pm 0.5 \times 10^7$ and $2.8 \pm 0.2 \times 10^6$ dm^3 mol^{-1} s^{-1} respectively. At very low oxygen concentrations, the first order IET was still observed. Even at a high concentration of oxygen, there is a residual absorption (in a few microseconds) observed at 410 nm, indicating that a fraction of nitro radicals (410 nm) are formed directly by $CO_2^{\bullet-}$ reaction.

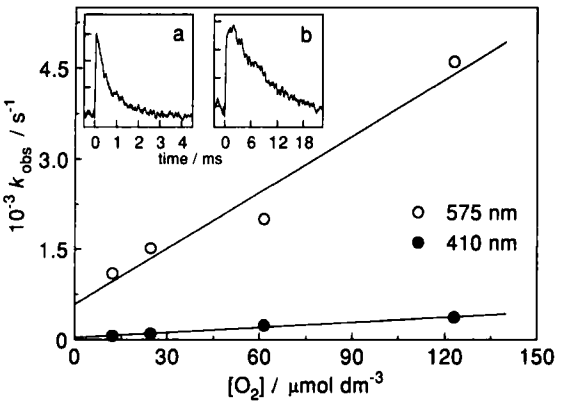


FIGURE 5 Linear plots showing the dependence of the observed rate of decay at 575 nm (o) and 410 nm (●) on oxygen concentration. Inset: Absorption-time plot at (a) 575 nm and (b) 410 nm in presence of 2% oxygen.

Electron Transfer Between RB91724 and Nitroimidazole

To verify whether intermolecular electron transfer is feasible between mono *N*-oxides and nitroimidazoles in solution we studied the electron transfer reaction between *N*-oxide radicals of RB91724 (donor) with the 2-nitroimidazole, misonidazole (acceptor) at pH 7. The reduction was initiated by $CO_2^{\bullet-}$ radicals and the concentration differential between *N*-oxide and misonidazole was adjusted ([RB91724] = 800 μmol dm^{-3} and [misonidazole] = 25–150 μmol dm^{-3}) such that initially mainly the *N*-oxide is reduced to its one-electron reduced intermediate and these radicals in turn react with misonidazole, by electron transfer. The decay of the *N*-oxide radicals was followed at 550 nm; the rate increased linearly with misonidazole concentration, and the bimolecular rate constant obtained from the slope of the linear plot was $3.2 \pm 0.2 \times 10^8$ dm^3 mol^{-1} s^{-1}. This rate constant clearly indicates that the energetics is favourable for electron transfer from the *N*-oxide radicals to the nitroimidazole.

Steady State Radiolysis and Loss of the Parent Drug

Steady-state γ-radiolysis studies of RB90745 (~50 μmol dm^{-3}) were carried out at different pH in N_2O saturated/formate solutions. Because a

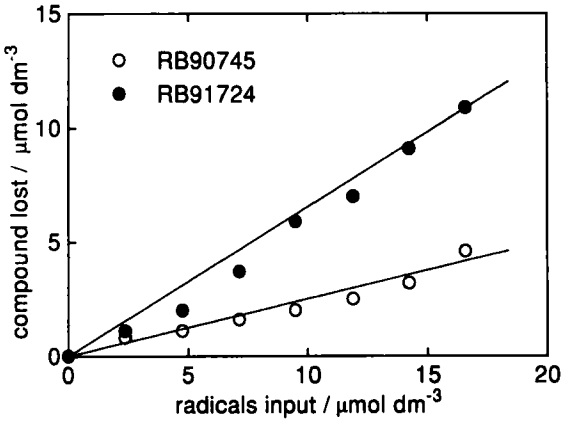


FIGURE 6 Loss of the parent compound as a function of radicals input, after steady-state radiolysis and separation by HPLC. Dose rate 6.9 Gy/min. (o) RB90745 and (●) RB91724.

mixture of products was observed, we followed the loss of the parent (by hplc) as a function of absorbed dose and pH. Figure 6 shows a linear plot for the loss of the parent of RB90745 at pH 7.4 as a function of absorbed dose or radical concentration; the data for RB91724 are also included at the same pH. From the slopes of these plots it can be seen that with RB91724 more than 0.5 molecules of parent were lost for one radical produced, whereas data for RB90745 indicate loss of ~0.25 molecules for one radical. i.e. four radicals are required to reduce one molecule of RB90745.

DISCUSSION

The bifunctional bioreductive drug RB90745 has an imidazo[1,2,-*a*]quinoxaline mono *N*-oxide function and a nitro heterocycle separated by an alkyl spacer. Earlier we studied the reduction chemistry of the quinoxaline mono *N*-oxides like RB91724.[14] They reacted with $CO_2^{\bullet-}$ at near the diffusion controlled limit. The radicals showed two prominent absorption bands in the 400 nm region and at 500–600 nm region. The pH dependent absorption changes yielded a pK_a value of 7.4 ± 0.1 for the unsubstituted mono *N*-oxide RB91724. Under anaerobic conditions the mono *N*-oxide radicals decayed by second order kinetics by radical-radical reactions. The radicals were quenched by oxygen with rate constants of 1–3 × 10^8 dm^3 mol^{-1} s^{-1}.

Nitroimidazoles like misonidazole react with $CO_2^{\bullet-}$ at the diffusion controlled limits, the radical anions absorbing at 400 nm with pK_a of 5.7–6.1.[15] The radicals at low concentration decay by first order kinetics and at high dose by second order kinetics. They are quenched by oxygen with rate constants of 10^6–10^7 dm^3 mol^{-1} s^{-1}.

Initial attack by the $CO_2^{\bullet-}$ radical on RB90745 is expected to be approximately equally probable with the nitro group and the *N*-oxide function, as the reactivity of the two individual compounds with $CO_2^{\bullet-}$ is comparable. The absorption at ~400 nm on the initial time scales (microseconds) may be the net absorption by the *N*-oxide radicals, the nitro radicals and the bleaching of the parent compound. However the absorption at 550–600 nm region may correspond to the radicals of the *N*-oxide only, since 2-nitroimidazole radicals usually show little absorption in this region. Subsequent to the initial reduction, the absorption signal at 550 nm decayed completely in a few milliseconds with a simultaneous increase in the absorption at 400–410 nm, suggesting an intramolecular transformation: an IET from the *N*-oxide function to the nitro group. The prototropic equilibria of the radicals measured at 590 nm and 410 nm gave pK_a values of 4.3 and 7.6, and 5.6 respectively. Comparing these pK_as with those of the radicals of the nitroimidazoles and the *N*-oxides,[14,15] we can conclude that the radical absorbing in the 550–600 nm region, with a pK_a of 7.6 is an *N*-oxide radical and the radical formed after a few milliseconds, absorbing at 410 nm with a pK_a of 5.6 is a nitro radical.

Studies on the reactivity of the radicals of RB90745 with oxygen gave two different rate constants. The radicals absorbing at 410 nm react with oxygen at an order of magnitude slower rate than the radicals at 550–600 nm. The oxygen quenching rate constant determined at 410 nm is comparable to that of misonidazole.[15] However, the radicals at

550–600 nm corresponding to the *N*-oxide function appear slightly less reactive towards oxygen than most of the mono-*N*-oxides (assuming no significant change in rate constant with pH). Too high a rate constant with oxygen in RB91724 was one of the reasons thought responsible for the poor *in vivo* efficacy of the drug, except in extreme hypoxia.[14] RB90745 appears slightly more effective than RB91724, which may be due to the reduction in the overall reactivity of the *N*-oxide radical with oxygen.

The potential difference between the *N*-oxides and nitro compounds indicated the possibility of electron transfer between the two groups. The one-electron reduction potentials of imidazo[1,2-*a*]quinoxaline mono *N*-oxides were estimated to be ~ –0.7 to –0.8 V vs. NHE using viologens and 1-methyl nicotinamide as standards.[14] The one-electron reduction potentials of the nitro imidazoles are –0.4 to –0.5 V.[15,16] Thus difference in the reduction potentials is of the order of –0.3 to –0.4 V. The net driving force for this reaction is therefore < –0.5 V. Due to this small free energy change a slow IET is expected from the *N*-oxide to the nitro function.[17,18] The rates of electron transfer are dependent on the distance between the two partners and may change with the length of the spacer. By constructing molecular models, the spacer length was determined to be ~1 nm.

The likelihood of competition between IET and electron transfer to oxygen of radical centres on the *N*-oxide function may be calculated. Thus in hypoxic cells with $[O_2]$ ~ 5 μmol dm^{-3}, *N*-oxide radicals react with oxygen on a time scale of $k[O_2]$ ~ $3.1 \times 10^7 \times 5 \times 10^{-6}$, ~ 1.5×10^2 s^{-1}, inefficiently competing with IET at ~10^3 s^{-1} at pH ~ 7.4. Intermolecular electron transfer with a rate constant of 3×10^8 dm^3 mol^{-1} s^{-1} was observed between RB91724 and misonidazole at pH 7, faster than the reactions of the radical centres with oxygen.

Protonation is likely to influence the kinetics of intramolecular electron transfer. At pH values between the p*K* values of the two radical sites (i.e. ~5.6–7.6), the energy gap between donor and acceptor might decrease by ~0.06 V per pH unit, a relatively small change in the overall energy gap of ~0.3–0.4 V. Protonated species often react much slower than their deprotonated conjugates, but a full appreciation of the effects of pH on IET is beyond the scope of this paper.

The steady-state radiolysis results and loss of the parent with RB90745 suggest that four radicals are required to reduce one parent molecule (four electron stoichiometry). Earlier we investigated in detail radiolysis and product distribution of the mono and di-*N*-oxides at different pH values. A significant feature of the radiolytic reduction of the *N*-oxides in the presence of H-donors such as formate is a chain reaction for the loss of the drug. As two radicals are required to produce any stable product (for a two electron stoichiometry), we expect loss of one parent molecule for the input of two radicals, or 0.5 molecules are expected to be lost for one radical, whereas we observed loss of four molecules for the input of two radicals at pH <pK_a. The radicals of the *N*-oxide are oxidising in nature and abstract hydrogen from hydrogen donors. In 0.1 mol dm^{-3} formate, it is assumed that the *N*-oxide radicals abstract hydrogen from the formate and generate another $CO_2^{\bullet-}$ radical. This radical in turn can reduce another drug molecule in its vicinity causing a small chain reaction. The chain reaction is absent at high pH, as the protonated radicals are more important in abstracting hydrogen than their conjugates. Thus at pH >7, most of the *N*-oxides show two electron stoichiometry.

In general nitro compounds are reduced to the nitroso compounds initially and are further reduced to hydroxylamines.[19] The formation of hydroxylamines by the reduction of nitro compounds is a four-electron process, *i.e.* requiring four radicals to reduce one parent molecule. The absence of a chain process even at low pH and the four electron stoichiometry for RB90745, indicate reduction chemistry similar to the nitro compounds but not of the *N*-oxides. This further supports our findings that following the initial reduction by $CO_2^{\bullet-}$, an IET takes place from the *N*-oxide function to the nitro moiety and the

compound shows characteristic reduction chemistry of nitro compounds.

The precise mode of drug action is unknown although it has been suggested that the mechanism may involve oxidation of sugar radicals in DNA produced by the *N*-oxide radicals involving hydrogen abstraction. Using model hydrogen donors like deoxy-ribose, the hydrogen abstraction rates were estimated to be $\sim 10^2 - 10^3$ dm^3 mol^{-1} s^{-1} for some mono and di-*N*-oxides.[14,20] For RB90745, the IET rates ($\sim 10^3$ s^{-1}) may be of the same order as the hydrogen abstraction rates and hence would compete with them. Since hydrogen abstraction is a bimolecular process, it may be prevented completely at low concentration of H-donors. Thus the IET may reduce the cytotoxicity of the drug would otherwise have shown compared to monofunctional derivatives.

These results are relevant to the design of new bifunctional anti-tumour drugs, where two different functional groups with different mechanisms of bioreductive action are attached. To increase the toxicity of the drug, it is necessary to prevent all other competing processes like IET. Since IET rates are influenced by the driving force (difference in the reduction potential) and the distance between the two groups, it is possible to vary toxicity by either of the two methods: increasing the length of the spacer to slow down the IET or by decreasing the potential difference between the two functions. In general it has been observed that the di-*N*-oxides are more electron -affinic (e.g. the reduction potential of tirapazamine is – 0.45 V v. NHE)[14] than the mono *N*-oxides. Therefore it might be advantageous to link the di-*N*-oxides to nitroimidazoles to exploit the bifunctional nature of the drugs. These types of compounds may be more effective anti-cancer drugs with the combined properties of radiosensitisers and hypoxia specific toxins.

Acknowledgements

The authors are grateful to Drs L.P. Candeias and S.A. Everett for useful discussions and to Dr B. Vojnovic and his team for the continuing development of the pulse radiolysis facility. This work was supported by the European Communities 'Marie Curie' research fellowship, the Cancer Research Campaign [CRC] and Medical Research Council. KIP thanks the Department of Science and Technology, Government of India for its support.

References

1. G.E. Adams and I.J. Stratford (1994) Bioreductive drugs for cancer therapy: the search for tumor specificity, *International Journal of Radiation Oncology Biology and Physics*, **29**, 231–238.
2. K. Laderoute, P. Wardman and A.M. Rauth (1988) Molecular mechanisms for the hypoxia-dependent activation of 3-amino-1,24-benzotriazine-1,4-dioxide (SR4233). *Biochemical Pharmacology*, **37**, 1487–95.
3. M.A. Naylor (1994) Novel N-oxides as bioreductive drugs. *Oncology Research*, **6**, 483–491.
4. M.A. Naylor, M.A. Stevens, J. Nolan, B. Sutton, J.H. Tocher, E.M. Fielden, G.E. Adams and I.J. Stratford (1993) Heterocyclic mono N-oxides with potential applications as anti-tumor drugs: Part 1. 8-alkyl amino-substituted phenylimidazo [1,2-*a*] quinoxalines. *Anti-Cancer Drug Design*, **8**, 439–461.
5. M.A. Naylor, B.M. Sutton, J. Nolan, P. O'Neill, E.M. Fielden, G.E. Adams, and I.J. Stratford (1994) Radiolytic and photochemical reduction of the hypoxic cytotoxin 1,2-dihydro-8-(4-methylpiperazinyl)-4-phenylimidazo [1,2-*a*] pyrido [3,2-*e*] pyrazine 5-oxide (RB90740) and a potential mechanism for hypoxia-selective toxicity. *International Journal of Radiation Oncology Biology and Physics*, **29**, 333–337.
6. J.M. Brown (1993) SR4233 (Tirapazamine): a new anticancer drug exploiting hypoxia in solid tumours. *British Journal of Cancer*, **67**, 1163–1170.
7. E.M. Zeman, M.A. Baker, M.J. Lemmon, C.I. Pearson, J.A. Adams, J.M. Brown, W.W. Lee and M. Tracy (1989) Structure activity relationships for benzotriazine di-*N*-oxides. *International Journal of Radiation Oncology Biology and Physics*, **16**, 977–81.
8. E.M. Zeman, J.M. Brown, M.J. Lemmon, V.K. Hirst and W.W. Lee (1986) SR-4233: a new bioreductive agent with high selective toxicity for hypoxic mammalian cells. *International Journal of Radiation Oncology Biology and Physics*, **12**, 1239–42.
9. P.L. Olive, R.E. Durrand and D.J. Chaplin (1987) Cytotoxicity of RSU 1069 in spheroids and murine tumours. *International Journal of Radiation Oncology Biology and Physics*, **13**, 1361–1366.
10. A.R.J. Silver, P. O'Neill and T.C. Jenkins (1985) Induction of DNA Strand Breaks by RSU-1069, A nitroimidazole-aziridine radiosensitiser. *Biochemical Pharmacology*, **34**, 3537.
11. L.P. Candeias, S.A. Everett and P. Wardman (1993) Free radical intermediates in the oxidation of flavone-8-acetic acid: possible involvement in its anti-tumor activity. *Free Radical Biology and Medicine*, **15**, 385–94.
12. J.W.T. Spinks and R.J. Woods (1990) *Introduction to Radiation Chemistry*, 3rd edition, John Wiley & Sons, New York.
13. Q.G. Mulazzani, M. D'Angelantonio, M. Venturi, M.Z. Hoffman and M.A.J. Rodgers (1986) Interaction of formate and oxalate ions with radiation-generated radicals in aqueous solution. Methyl viologen as a mechanistic probe, *Journal of Physical Chemistry*, **90**, 5347–55.

14. P. Wardman, K.I. Priyadarsini, M.F. Dennis, S.A. Everett, M.A. Naylor, K.B. Patel, I.J. Stratford, M.R.L. Stratford and M. Tracy (1996) Chemical properties which control selectivity and efficacy of aromatic *N*-oxide bioreductive drugs, *British Journal of Cancer*, **73**, (Suppl. XXVII), in press.
15. P. Wardman (1985) Some reactions and properties of nitro radical-anions important in biology and medicine, *Environmental Health Perspectives*, **63**, 101–112.
16. D. Meisel and P. Neta (1975) One-electron reduction potentials of nitro compounds and radiosensitizers correlation with spin densities and their radical anions, *Journal of the American Chemical Society*, **97**, 5198–5203.
17. G.L. Cross and J.R. Muller (1988) Intramolecular long range electron transfer in organic molecules. *Science*, **240**, 440.
18. S.S. Isied, M.Y. Ogawa and J.F. Wishart (1992) Peptide-mediated intramolecular electron transfer: Long-range distance dependence. *Chemical Reviews*, **92**, 381–94.
19. R.A. McClelland, J.R. Fuller, N.E. Seaman, A.M. Rauth and R. Battistella (1984) 2-Hydroxylaminoimidazoles – unstable intermediates in the reduction of 2-nitroimidazoles. *Biochemical Pharmacology*, **33**, 303–309.
20. P. Wardman, M.F. Dennis, S.A. Everett, K.B. Patel, M.R.L. Stratford and M. Tracy (1995) Radicals from one-electron reduction of nitro compounds, aromatic *N*-oxides and quinones: the kinetic basis for hypoxia-selective, bioreductive drugs. In *Free Radicals and Oxidative stress: Environment, Drugs and Food Additives*. (eds. C. Rice-Evans, B. Halliwell, and G.G. Lunt), (*Biochemical Society Symposium* **61**), Portland Press, London, pp. 171–94.