J|A|C|S
ARTICLES
Published on Web 12/13/2005

Potentiation of the Cytotoxicity of the Anticancer Agent Tirapazamine by Benzotriazine *N*-oxides: The Role of Redox Equilibria

Robert F. Anderson,*[†,‡] Sujata S. Shinde,[†,‡] Michael P. Hay,[‡] and William A. Denny[‡]

Contribution from the Department of Chemistry and Auckland Cancer Society Research Centre, The University of Auckland, Private Bag 92019, Auckland 1, New Zealand

Received September 4, 2005; E-mail: r.anderson@auckland.ac.nz

Abstract: Tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide), the lead bioreductive drug with selective toxicity for hypoxic cells in tumors, is thought to act by forming an active oxidizing radical of high one-electron reduction potential, *E*(1), when reduced by reductases. It has a dual mechanism of action, both generating DNA radicals, following its one-electron reduction and subsequently oxidizing these DNA radicals to form labile cations or hydrolyzable lactones through transferring an O atom, resulting in DNA strand breaks. These parallel secondary reactions have been proposed to be also initiated by its two-electron reduced metabolite, the 1-oxide. We have used pulse radiolysis to show that the benzotriazinyl radical of a highly soluble analogue of tirapazamine, the 3-(*N*,*N*-dimethyl-1,2-ethanediamine) analogue, is able to oxidize tirapazamine itself. We have found that both tirapazamine and the 1-oxides are in equilibrium with their respective benzotriazinyl radicals, with high concentrations of the more soluble 1-oxide maintaining a high concentration of the more reactive oxidizing radical of tirapazamine. The one-electron reduction potentials, *E*(1), of the 1-oxides and related compounds have been measured and, together with the *E*(1) values of tirapazamine and the 2-nitroimidazole radiosensitizer, misonidazole, are shown to predict the published percentages of electron transfer. This radical chemistry study gives an insight into the mechanisms of the potentiation of radical damage, reported for DNA, that underlies the hypoxic cytotoxicity of electron affinic compounds. The *E*(1) values of the benzotriazinyl radicals of the benzotriazine compounds govern the position of the redox equilibria, which determine the amount of initial radical damage. The *E*(1) values of the 1,4-dioxides and 1-oxide compounds govern the degree of potentiation of the initial radical damage once formed.

Introduction

There is currently much interest in exploiting tumor hypoxia to produce cytotoxins from prodrugs which selectively attack chemo- and radioresistant hypoxic tumor cells.[1] The drug, tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide, **1**), is the most clinically advanced bioreductive prodrug using this strategy. [2,3] Both the known major two-electron metabolite (the 1-oxide, **4**), and the four-electron reduced metabolite (noroxide, **6**) of **1** are relatively nontoxic to hypoxic cells,[4] and research efforts directed to understanding the cytotoxicity of **1** have mainly focused on the species formed following its one-electron reduction. There is universal agreement that the oxygen-sensitive, one-electron reduction of **1** by reductases under hypoxia leads to the formation of an active radical species that induces DNA damage leading to strand breaks and that such lesions underlie the cytotoxicity of **1**.[5−8] Initial one-electron reduction of **1** results in its reducing radical anion, **2**/**3**,[9] and it has been proposed that this species subsequently eliminates the •OH radical as the oxidizing species.[10] We have presented both spectral and kinetic evidence that **3** undergoes a transformation to the benzotriazinyl radical, **5**, Scheme 1, which is an oxidizing species of one-electron reduction potential $E(\mathbf{5}/\mathbf{4}) = 1.31$ V, capable of causing radical damage on DNA,[11,12] and that this redox parameter tracks the hypoxic cytotoxicity to mammalian cells of a series of analogues of **1**.[13]

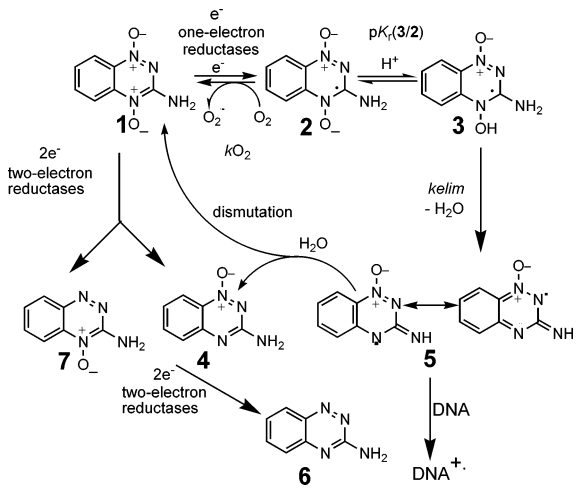
1 has also been shown to oxidize the initial radical damage to components of DNA through the formation and breakdown of adducts producing one-electron-reduced species, **2**/**3**, and in

† Department of Chemistry, The University of Auckland.
‡ Auckland Cancer Society Research Centre, The University of Auckland.

(1) Brown, J. M.; Wilson, W. R. *Nat. Rev. Cancer* **2004**, *4*, 437−447.
(2) von Pawel, J.; et al. *J. Clin. Oncol.* **2000**, *18*, 1351−1359.
(3) Rischin, D.; Peters, L.; Fisher, R.; Macann, A.; Denham, J.; Poulsen, M.; Jackson, M.; Kenny, L.; Penniment, M.; Corry, J.; Lamb, D.; McClure, B. *J. Clin. Oncol.* **2005**, *23*, 79−87.
(4) Baker, M. A.; Zeman, E. M.; Hirst, V. K.; Brown, J. M. *Cancer Res.* **1988**, *48*, 5947−5952.
(5) Brown, J. M. *Br. J. Cancer* **1993**, *67*, 1163−1170.
(6) Biedermann, K. A.; Wang, J.; Graham, R. P.; Brown, J. M. *Br. J. Cancer* **1991**, *63*, 358−362.
(7) Wang, J.; Biedermann, K. A.; Brown, J. M. *Cancer Res.* **1992**, *52*, 4473−4477.
(8) Delahoussaye, Y. M.; Evans, J. W.; Brown, J. M. *Biochem. Pharmacol.* **2001**, *62*, 1201−1209.
(9) Laderoute, K.; Wardman, P.; Rauth, A. M. *Biochem. Pharmacol.* **1988**, *37*, 1487−1495.
(10) Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1996**, *118*, 3380−3385.
(11) Anderson, R. F.; Shinde, S. S.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 748−756.
(12) Shinde, S. S.; Anderson, R. F.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *J. Am. Chem. Soc.* **2004**, *126*, 7865−7874.
(13) Anderson, R. F.; Shinde, S. S.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *Org. Biomol. Chem.* **2005**, *3*, 2167−2174.

10.1021/ja0559101 CCC: $33.50

Scheme 1. Reduction of Tirapazamine



the case of the oxidation of sugar radicals, a labile sugar cation.[12,14−16] In addition to this electron-transfer event, it has been shown, both experimentally in a model system[14,16] and theoretically,[17] that an O-atom from the *N*-oxide moiety of **1** can be donated to the radical site on the deoxyribose of DNA. Both the oxidation of carbon-centered radicals by electron-affinic compounds to form labile cations (as the basis of radiosensitization[18]) and the transfer of an O atom (which fixes the radical damage[19]) are presumed to be cytotoxic events. Recently, it has been reported that the hypoxic cytotoxicity of **1** to mammalian cells can be increased through the co-administration of high concentrations of its 1-oxide metabolite, **4**, or its more water-soluble form, the 3-(*N*,*N*-dimethyl-1,2-ethanediamine) analogue, **8**.[20] The mechanism of this potentiation appears to be different from that of the nitroimidazole radiosensitizers, misonidazole and metronidazole, that act as DNA radical oxidants, since similarly high concentrations of these compounds do not act as potentiators of the cytotoxicity of **1**. In this study we further examine the redox properties of **1** and of the benzotriazine mono-*N*-oxide derivatives **4** and **8**, as well as the interactions between their radical forms, to help gain a fuller understanding of the observed potentiation of cytotoxicity as well as the molecular mechanisms of cytotoxicity and radiosensitization.

Results

Benzotriazinyl radicals (e.g. **5**) are rapidly produced upon the one-electron oxidation of their 1-oxide analogues by the $SO_4^{\bullet-}$ radical.[11] The benzotriazinyl radicals **5** and **9** (the 3-(*N*,*N*-dimethyl-1,2-ethanediamine) analogue of **5**) were found to oxidize the redox indicator 1,2,4-trimethoxybenzene, TMB, to different extents. Whereas near complete electron transfer from TMB to **5**, to form its one-electron oxidized form, the radical $TMB^{+\bullet}$, is expected and observed, since $E(TMB^{+\bullet}/TMB) = 1.13$ V[21] and $E(\mathbf{5}/\mathbf{4}) = 1.31$ V,[11] an equilibrium 1 was established on the 200-μs time scale between the one-electron-oxidized forms of **8** and TMB, species **10** and $TMB^{+\bullet}$.

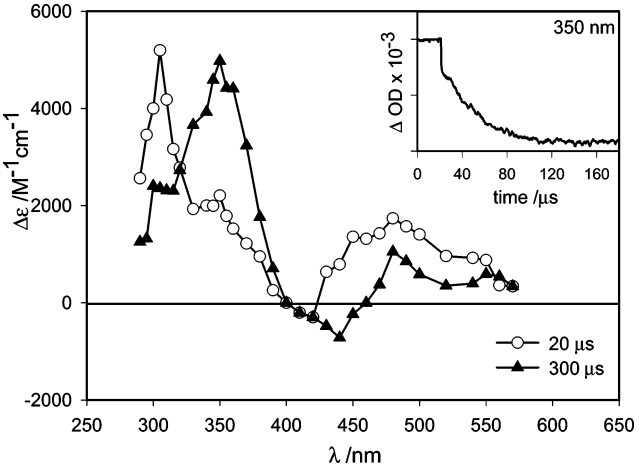


Figure 1. Absorption difference spectra of radicals, relative to those of parent compound, at (○) 20 μs and (▲) 300 μs following pulse radiolysis (2.5 Gy) of **8** (300 μM) in N_2-saturated solutions containing potassium peroxodisulfate (15 mM), 2-methyl-2-propanol (0.2 M) at pH 7.0. (Insert) Oscilloscope trace of the change in transmittance following pulse radiolysis.

$$TMB^{+\bullet} + \mathbf{8} \overset{K_1}{\rightleftharpoons} TMB + \mathbf{10} + H^+ \qquad (1)$$

The equilibrium constant K_1 of 0.35 ± 0.18, determined from absorbance changes at 460 nm for three mixtures varying the concentrations of TMB and **8** (0.1−1.0 mM), gives a calculated value of $E = 1.18 \pm 0.02$ V. This is somewhat lower than previous reported 1.27 ± 0.01 V using 1,4-dimethoxybenzene, DMB, as the redox indicator ($E(DMB^{+\bullet}/DMB) = 1.30$ V[21]) where equilibrium was established on a much shorter time scale.[12] On close examination it was observed that an initial radical, **9**, is formed by the reaction of the $SO_4^{\bullet-}$ radical with **8**, which exhibits a similar absorption spectrum to that seen on the oxidation of **4**, and undergoes a fast transformation ($k = 1.0 \times 10^4$ s^{-1}at pH 7), independent of the concentration of **8,** to form the radical, **10**, with an absorbance band centered near 350 nm. The absorption spectra of **9** and **10** are presented in Figure 1.

As such a transformation is not observed with **4**, or for compounds with 3-NHR substituted side chains that do not contain the dimethylamine moiety (data not shown), we interpret this as an intramolecular rearrangement of the initially formed benzotriazinyl radical on the aromatic ring system, to form a radical of lower one-electron reduction potential, involving the side-chain amine. The formation of radical **10** from **9** occurs above pH 4 with an apparent pK_a of 6.10 ± 0.18, Figure 2. The rate constant for this reaction increases with pH, plateauing to ca. 5×10^4 s^{-1} above pH 10. As is common for oxidizing radicals, **10** was found not to react with O_2.

The $SO_4^{\bullet-}$ radical was found also to oxidize **1** at $5.0 \pm 2.0 \times 10^9$ M^{-1} s^{-1} to produce the spectrum presented in Figure 3, and a redox equilibrium 2 between this new radical species, **11**, and TMB was established.

$$TMB^{+\bullet} + \mathbf{1} \overset{K_2}{\rightleftharpoons} TMB + \mathbf{11} \qquad (2)$$

From the measured K_2 of 1.02 ± 0.07, a value for $E(\mathbf{11}/\mathbf{1}) = 1.15 \pm 0.01$ V is calculated. On thermodynamic grounds,

(14) Jones, G. D. D.; Weinfeld, M. *Cancer Res.* **1996**, *56*, 1584−1590.
(15) Daniels, J. S.; Gates, K. S.; Tronche, C.; Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1254−1257.
(16) Hwang, J.-T.; Greenberg, M. M.; Fuchs, T.; Gates, K. S. *Biochemistry* **1999**, *38*, 14248−14255.
(17) Ban, F.; Gauld, J. W.; Boyd, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 7320−7325.
(18) Wardman, P. *Radiat. Phys. Chem.* **1987**, *30*, 423−432.
(19) Kappen, L. S.; Lee, T. R.; Yang, C.-C.; Goldberg, I. H. *Biochemistry* **1989**, *28*, 4540−4542.
(20) Siim, B. G.; Pruijn, F. B.; Sturman, J. R.; Hogg, A.; Hay, M. P.; Brown, J. M.; Wilson, W. R. *Cancer Res.* **2004**, *64*, 736−742.

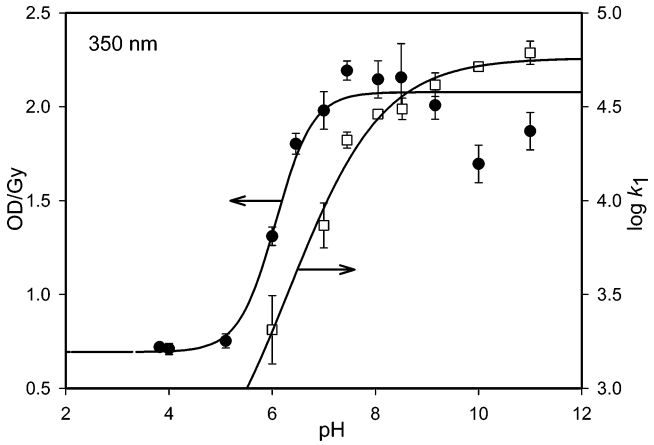


Figure 2. Changes in absorption (●) and in the rate constant (□) for the formation with pH of the transient species centered at 350 nm, following pulse radiolysis of the solution in Figure 1.

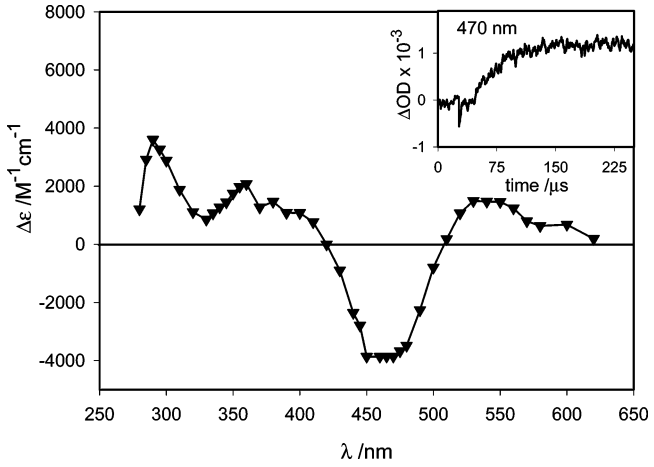


Figure 3. Absorption difference spectrum of the radical, relative to parent compound, at (▼) 200 μs following the pulse radiolysis (2.5 Gy) of **1** (150 μM) in N_2-saturated solution containing potassium peroxodisulfate (15 mM), 2-methyl-2-propanol (0.2 M) at pH 7.0. (Insert) Oscilloscope trace of the change in transmittance at 470 nm following pulse radiolysis under the same conditions for a mixture of **8** (500 μM) and **1** (100 μM).

the radicals **5**, **9**, and **10** are expected to oxidize **1** to radical **11**, as well as forming an equilibrium between **11** and **8**. The slow formation of the benzotriazinyl radical **5** (~100 s^{-1} [11]), following the one-electron reduction of **1**, and the poor solubility of **4** which can be oxidized to radical **5** by the $SO_4^{\bullet-}$ radical prevented the pulse radiolysis investigation of the reaction of radical **5** with **1** which requires approximately millimolar concentrations to quickly establish redox equilibria. However, the higher solubility of **8** enabled it to be used to produce radical **10** and to study its reaction with **1**, equilibrium 3.

$$\mathbf{10} + \mathbf{1} + H^+ \overset{K_3}{\rightleftharpoons} \mathbf{8} + \mathbf{11} \qquad (3)$$

The data obtained for three mixtures of **8** and **1** were used to calculate K_3 as 3.50 ± 0.97, and hence ΔE (E(**10**, H^+/**8**) − E(**11**/**1**)) = 0.040 V, consistent with the difference in one-electron reduction potentials obtained above.

Similarly, employing redox equilibria to determine one-electron reduction potentials, E(1), we have used methyl viologen, MV^{2+}, to measure a value for the benzotriazine 4-oxide, **7**, and triquat, TQ^{2+}, for the 2-oxide analogue, the noroxide, **6**, and the related quinoxaline 1,4-dioxide. These data, along with the one-electron reduction potentials of the radical species, are presented in Table 1.

Table 1. One-Electron Reduction Potentials

benzotriazine 1,4 dioxide A	E(A/A$^{\bullet-}$)/V[b]	E(A$^{\bullet}$,H$^+$/A)/V[b]
3-$NH_2$1,4-dioxide	−0.456[c,d] (**1**)	1.15[g](**11**)
benzotriazine *N*-oxide, B	**E(B/B$^{\bullet-}$)/V[b]**	**E(B$^{\bullet}$,H$^+$/B)/V[b]**
3-NH_2 1-oxide	−0.568[e](**4**)	1.31[f](**5**)
3-NHR[a]1-oxide	−0.502[e](**8**)	1.27[e](**9**)
		1.18[g](**10**)
3-NH_2 4-oxide	−0.378[g](**7**)	1.14[g]
3-NH_2 2-oxide	−0.650[g]	1.19[g]
3-NH_2 noroxide	−0.561[g](**6**)	1.20[g]
quinoxaline, C	**E(C/C$^{\bullet-}$)/V[b]**	
1,4-dioxide	−0.574[g]	

[a] R = $CH_2CH_2N^+H(CH_3)_2$. [b] Error ± 0.01 V. [c] Reference 22. [d] Reference 23. [e] Reference 12. [f] Reference 11. [g] This work.

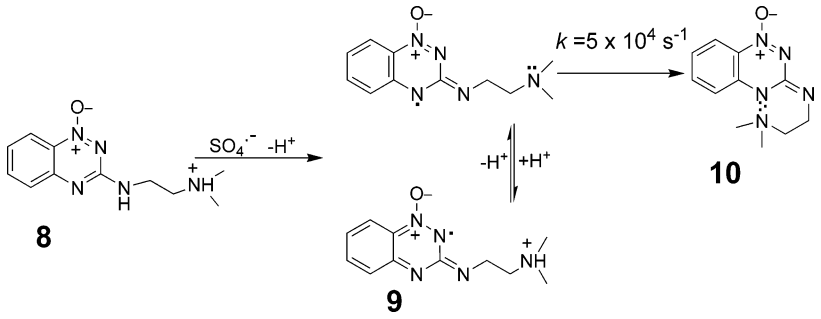
Discussion

The above results demonstrate that benzotriazinyl radicals derived from benzotriazine oxides undergo complex reactions. A new transient species, **10**, is formed following the formation of benzotriazinyl radical **9** via an intramolecular process involving the *N*,*N*-dimethylamine moiety of the 3-NHR alkyl side chain. Electron transfer from the nonbonding pair electrons of the *N*,*N*-dimethylamine moiety to the benzotriazinyl radical, forming a *N*-radical cation, is a possible mechanism. The calculated pK_a of the side-chain amine of **8** is 8.65 ± 0.28 (ACD pK_a calculator, v7.1, ACD Inc, Toronto Canada). This is expected to decrease on formation of the benzotriazinyl radical, which could account for the apparent shift in pK_a to 6.1, Figure 2. Similar removal of an electron from trimethylamine results in the formation of a *N*-radical cation with an optical absorption spectrum centered near 260 nm (ϵ = 3300 M^{-1} cm^{-1}) due to transition of the unpaired p electron.[24] Thus, the grow-in of the large absorption band centered near 350 nm (ϵ = ~5500 M^{-1} cm^{-1}) cannot be accounted for by a shift of the radical from the benzotriazinyl ring to the side chain. The position, width, and intensity of the new band is reminiscent of $\sigma-\sigma^*$ absorption bands arising from three-electron-bonded radicals known to be formed between heteroatoms.[25−28] In these systems a geometrically favorable overlap of a nonbonding p-orbital pair of electrons on one atom with an unpaired p-electron of another atom is thought to form a $2\sigma-1\sigma^*$ three-electron bond in an intramolecular process.[29] While neutral S−N three-electron-bonded species[28,30,31] and $N-N^+$ three-electron-bonded cationic species are known,[32−34] we believe **10** may well be the first

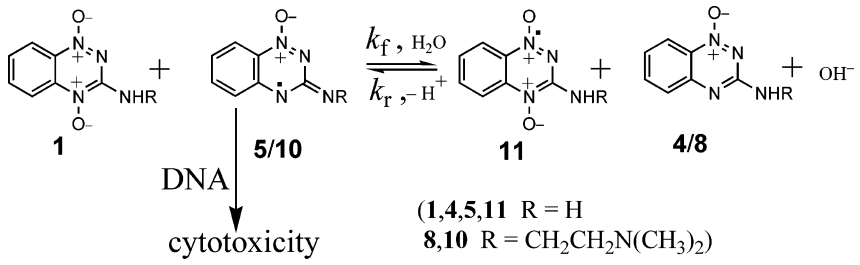
(21) Literature value is 1.13 ± 0.02 V (Jonsson, M.; Lind, J.; Reitberger, T.; Eriksen, T. E.; Merenyi, G. *J. Phys. Chem.* **1993**, *97*, 11278−11282); however, we have redetermined this value as 1.15 ± 0.02 V against 1,4-dimethoxybenzene.

(22) Priyadarsini, K. I.; Tracy, M.; Wardman, P. *Free Radical Res.* **1996**, *25*, 393−400.
(23) Hay, M. P.; Gamage, S. A.; Kovacs, M.; Pruijn, F. B.; Anderson, R. F.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 169−182.
(24) Das, S.; Von Sonntag, C. *Z. Naturforsch.* **1986**, *41b*, 505−513.
(25) Gilbert, B. C.; Hodgeman, D. K. C.; Norman, R. O. C. *J. C. S. Perkin II* **1973**, 1748−1752.
(26) Bonifacic, M.; Mockel, H.; Bahnemann, D.; Asmus, K.-D. *J. C. S. Perkin II* **1975**, 675−685.
(27) Asmus, K.-D.; Bahnemann, D.; Fischer, C.-H.; Veltwisch, D. *J. Am. Chem. Soc.* **1979**, *101*, 5322−5329.
(28) Asmus, K.-D.; Gobl, M.; Hiller, K.-O. *J. C. S. Perkin II* **1985**, 641−646.
(29) Asmus, K.-D. *Acc. Chem. Res.* **1979**, *12*, 436−442.

Scheme 2. Formation and Rearrangement of Benzotriazinyl Radicals of **8**



Scheme 3. Redox Equilibrium



example of a neutrally charged N−N three-electron-bonded species. Formation of such a species is probably due in part to the benzotriazinyl radical being relatively stabilized by having the unpaired electron spread over more than one nitrogen atom, and the 3-NHR side-chain forming a six-membered ring, permitting favorable overlap of the lone pair of electrons on the dimethylamine, Scheme 2.

This study provides new insights into the mechanism by which high concentrations of benzotriazine mono *N*-oxides can potentiate the cytotoxicity of **1**. The benzotriazinyl radical **5**, produced following the one-electron reduction of **1**, Scheme 1, can react either with target DNA to produce radical damage on DNA, or with **1** itself in a competitive reaction to produce the less oxidizing **11** radical. In the presence of excess **8**, the redox equilibrium (Scheme 3) comes into play, effectively maintaining a higher concentration of the more oxidizing radicals (**5** and **10**) by driving the equilibrium toward the left-hand side with an expected increase in the damage on DNA, giving rise to cytotoxicity, Scheme 3.

However, the above scenario assumes that the concentration of **1** will be high enough to compete with the reaction of **5**/**10** with a target molecule such as DNA. This may well be the case when **1** is reduced by reductases in the cytoplasm of cells and diffusion to the nuclear target is compromised by the reaction of **5** with **1** in Scheme 3. The fact that it is possible to achieve high levels of **4** in vivo (ca. 50−80 μM), which results in an increase in the cytotoxicity of **1**,[20] supports the contention that the redox equilibrium, Scheme 3, is active in vivo.

Only the benzotriazine 1,4-dioxides, and not their mono *N*-oxide derivatives, undergo enzymatic one-electron reduction to form a radical which can damage DNA. However, both classes of compounds can potentiate the DNA damage.[14−16] The well-known dependency of radiosensitization on the one-electron reduction potential, $E(1)$, of electron-affinic compounds[36] is mirrored by the Hammett σ constants of substituents.[35] Similarly, compounds of higher electron affinity show both enhanced efficiency of electron transfer from DNA radicals in model systems, and enhanced DNA bond breakage by releasing phosphate from 5′-purine nucleotides[37] and the forming of their radical anions.[12] The extent of ^{16}O incorporation into 2-deoxyribonolactone from the *N*-oxide moieties of **1**, **4**, **7**, and quinoxaline 1,4-dioxide and from the nitro group of the 2-nitroimidazole, misonidazole, in the presence of solvent $H_2{}^{18}O$ has been taken as a measure of O-atom transfer from these compounds to the test C1′-nucleotide radical.[16] The inverse analysis of these data, namely the percentage of electron transfer from the C1′-nucleotide radical to the electron-affinic compounds, tracks the $E(1)$ values of the compounds measured in the present study, Figure 4.

As increased cytotoxicity and radiosensitization abilities qualitatively correspond to higher $E(1)$ values for these compounds,[23,38] we conclude that O-atom transfer is a far less cytotoxic event compared to electron transfer from the nucleotide radical to the oxidizing compounds. This study supports the contention that the oxidation of a sugar radical on DNA, by electron transfer to aromatic electron-affinic nitroarenes and *N*-oxide compounds, underlies the major component of both radiosensitization and cytotoxicity.

Experimental Section

Chemicals. All reagents used were of analytical grade. Sodium hydroxide, perchloric acid, and phosphate buffers were obtained from

(30) Musker, W. K.; Hirschon, A. S.; Doi, J. T. *J. Am. Chem. Soc.* **1978**, *100*, 7754−7756.
(31) Musker, W. K.; Surdhar, P. S.; Ahmad, R.; Armstrong, D. A. *Can. J. Chem.* **1984**, *62*, 1874−1876.
(32) Alder, R. W.; Sessions, R. B. *J. Am. Chem. Soc.* **1979**, *101*, 3651−3652.
(33) Nelsen, S. F.; Alder, R. W.; Sessions, R. B.; Asmus, K.-D.; Hiller, K.-O.; Gobl, M. *J. Am. Chem. Soc.* **1980**, *102*, 1429−1430.
(34) Alder, R. W.; Bonifacic, M.; Asmus, K.-D. *J. Chem. Soc., Perkin Trans. 2* **1986**, 277−284.
(35) Raleigh, J. A.; Chapman, J. D.; Borsa, J.; Kremers, W.; Reuvers, A. P. *Int. J. Radiat. Biol.* **1973**, *23*, 377−387.
(36) Adams, G. E.; Clarke, E. D.; Flockhart, I. R.; Jacobs, R. S.; Sehmi, D. S.; Stratford, I. J.; Wardman, P.; Watts, M. E.; Parrick, J.; Wallace, R. G.; Smithen, C. E. *Int. J. Radiat. Biol.* **1979**, *35*, 133−150.
(37) Raleigh, J. A.; Greenstock, C. L.; Whitehouse, R.; Kremers, W. *Int. J. Radiat. Biol.* **1973**, *24*, 595−603.
(38) Wardman, P.; Dennis, M. F.; Everett, S. A.; Patel, K. B.; Stratford, M. R. L.; Tracy, M. In *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*; Rice-Evans, C. A., Halliwell, B., Lund, G. G., Eds.; Portland Press: London, 1995; Vol. 61, p 171−194.

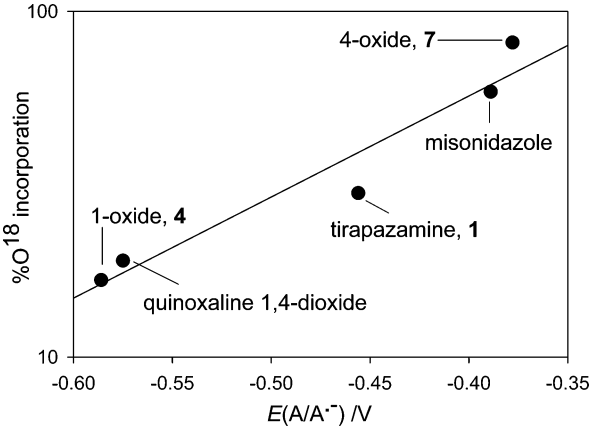


Figure 4. Dependence of the incorporation of ^{18}O from solvent $H_2{}^{18}O$ into 2′-deoxyribonolactone, following anaerobic photolysis of its precursor, on the one-electron reduction potential of the oxidizing compound. Incorporation data interpolated from ref 16.

Merck, and all other reagents were from Aldrich Chemical Co. All solutions were prepared in water purified by the Millipore "Milli-Q" system. Solution pH values were adjusted by the phosphate salts (5 mM) and either NaOH or $HClO_4$ when necessary.

Tirapazamine, **1**,[39] 1-oxide, **4**,[39] 2-oxide,[40] 4-oxide, **7**,[40] noroxide, **6**,[40] compound **8**,[12] and quinoxaline 1,4-dioxide[41] were synthesized as previously described.

Methods. Pulse radiolysis experiments were carried out at room temperature (22 ± 1 °C) using a 4 MeV linear accelerator, equipped with optical detection, to deliver a typical absorbed dose of 2−3 Gy in 200 ns.[42] The radiolysis of water produces three well-characterized reactive radical species and molecular products (concentrations in μM per absorbed dose of 1 Gy (J Kg^{-1}) given in parentheses).

$$H_2O \rightsquigarrow e^-_{aq}(0.28) + {}^\bullet OH(0.28) + H^\bullet(0.06) + H_2(0.04) + H_2O_2(0.07) + H_3O^+(0.28)$$

Rapid one-electron oxidation of the benzotriazine 1,4-dioxide, **1**, A, and the benzotriazine *N*-oxides, B, was carried out using the sulfate radical (E^0 $SO_4{}^{\bullet-}/SO_4{}^{2-}$ = ca. +2.5 V[43]) produced in N_2-saturated solution containing 0.2 M 2-methylpropan-2-ol and 20 mM peroxodisulfate. The radical yield, (G value) of $\mathbf{11}/\mathbf{B}^{+\bullet}$ in this systems is 0.28 μM Gy^{-1}.

$$e^-_{aq} + S_2O_8{}^{2-} \rightarrow SO_4{}^{\bullet-} + SO_4{}^{2-} \quad (4)$$

$${}^\bullet OH(H^\bullet) + (CH_3)_3COH \rightarrow {}^\bullet CH_2(CH_3)_2COH + H_2O(H_2) \quad (5)$$

$$SO_4{}^{\bullet-} + \mathbf{1}/B \rightarrow SO_4{}^{2-} + \mathbf{11}/B^{+\bullet} \quad (6)$$

The one-electron reduction potentials of the compounds were determined at pH 7.0 (5 mM phosphate buffer) by establishing redox equilibria between three mixtures of the one-electron reduced compounds and the reference compounds methyl viologen ($E(MV^{2+}/MV^{+\bullet})$ = −447 ± 7 mV) and triquat ($E(TQ^{2+}/TQ^{+\bullet})$ = −548 ± 7 mV)[44] and calculating ΔE values from the equilibrium constants, K_e, using the Nernst equation, as described in the literature.[45] Similarly, the one-electron reduction potentials of the radicals formed upon the one-electron oxidation of the compounds were determined using mixtures containing the reference compound 1,4-dimethoxybenzene ($E(DMB^{+\bullet}/DMB)$ = 1.30 ± 0.01 V) or 1,2,4-trimethoxybenzene ($E(TMB^{+\bullet}/TMB)$ = 1.15 ± 0.02 V).[21]

$$MV^{+\bullet}/TQ^{+\bullet} + B/C \overset{K_e}{\rightleftharpoons} MV^{2+}/TQ^{2+} + B^{\bullet-}/C^{\bullet-} \quad (7)$$

$$DMB^{+\bullet}/TMB^{+\bullet} + \mathbf{1}/B \overset{K_e}{\rightleftharpoons} DMB/TMB + \mathbf{11}/B^{+\bullet} \quad (8)$$

Acknowledgment. This work was supported by Grant 04/094 from the Health Research Council of New Zealand (R.F.A., S.S.S.), Grant CA082566-04 from the National Cancer Institute (M.P.H.), and the Auckland Division of the Cancer Society of New Zealand (W.A.D.). We thank G. Atwell for the synthesis and gift of quinoxaline 1,4-dioxide.

Supporting Information Available: Complete ref 2. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0559101

(39) Mason, J. C.; Tennant, G. *J. Chem. Soc. (B)* **1970**, 911−916.
(40) Fuchs, T.; Chowdhury, G.; Barnes, C. L.; Gates, K. S. *J. Org. Chem.* **2001**, *66*, 107−114.
(41) Copéret, C.; Adolfsson, H.; Khuong, T.-A. V.; Yudin, A. K.; Sharpless, K. B. *J. Org. Chem.* **1998**, *63*, 1740−1741.
(42) Anderson, R. F.; Denny, W. A.; Li, W.; Packer, J. E.; Tercel, M.; Wilson, W. R. *J. Phys. Chem. A* **1997**, *101*, 9704−9709.
(43) Eberson, L. *Adv. Phys. Org. Chem.* **1982**, *18*, 79−185.
(44) Stekhan, E.; Kuwana, T. *Ber. Bunsen-Ges. Phys. Chem.* **1974**, *78*, 253−259.
(45) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1755.