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Selective Interaction of Tirapazamine with DNA Bases and DNA

A Comparison of Cyclic Voltammetry and Electrolysis Techniques

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An electrochemical model has been used to study the reductive activation of the hypoxic cell cytotoxin tirapazamine (TPZ, 3-amino-1,2,4-benzotriazine-1,4-dioxide). Cyclic voltammetry and controlled potential electrolysis have been used to generate and study the 1-electron reduction product, the assumed biologically active species. Cyclic voltammetry of tirapazamine in dimethylformamide shows a quasi-reversible 1-electron reduction with the product showing a tendency to participate in a following chemical reaction. Controlled potential electrolysis to generate the 1-electron reduction product was unsuccessful due to the formation of a new redox-active species at less negative reduction potentials. However, the cyclic voltammetry of tirapazamine in the presence of *E. coli* DNA shows a decrease in the lifetime of the radical anion, signifying direct interaction with the DNA. The radical lifetime also decreased in the presence of adenine, thymine and guanine, but increased upon addition of cytosine and ribose. The study shows that cyclic voltammetry is an extremely useful tool for investigating the interaction between bio-reductive drugs and biological target molecules.

Keywords: Tirapazamine, reductive activation, cyclic voltammetry, electrosynthesis, DNA interaction

INTRODUCTION

Tirapazamine (TPZ, 3-amino-1,2,4-benzotriazine-1,4-dioxide, SR 4233) has successfully progressed to Phase III clinical trials as a hypoxia-selective anti-tumour agent where it has proved to be particularly effective when used in conjunction with cisplatin.[1,2] The selective toxicity of TPZ for hypoxic mammalian cells is due to the preferential reduction of the drug to cytotoxic metabolites under conditions of low oxygen tension, causing DNA damage and ultimately cell death.[3] The stable 2- and 4-electron reduction products, the mono- and zero-*N*-oxides, SR 4317 and SR 4330 respectively, show no significant biological activity, therefore, the proposed active species is the 1-electron reduction intermediate.[4,5] The high efficiency of TPZ to cause DNA damage is assigned to its reduction in the cell nucleus in close proximity to DNA, although the enzymes responsible have not been

identified.[6] Enzymatic reduction to yield the 1-electron reduction product resulted in radical-mediated oxidative DNA strand cleavage, with the drug acting as an oxygen mimetic.[7]

Electrochemical techniques are a highly effective way to study the charge-transfer properties of bio-reductive agents, including TPZ. Using controlled potential electrolytic reduction of TPZ in the presence of DNA, the drug-induced DNA damage can be studied to provide further information on the biological implications of the redox-activation step.[8] Voltammetric studies in aqueous buffer show that TPZ is reduced in a 2-stage process. The first step involves a 4-electron addition, resulting in loss of both *N*-oxide groups; the second step is assigned to a 2-electron reduction of the benzotriazine heterocycle. Changing the solvent to dimethylformamide or acetonitrile alters the reduction mechanism with up to six reduction steps being identified.[9] Despite this complexity the first reduction is sufficiently isolated that it can be studied without interference from further reduction steps. The first reduction is characterized as a quasi-reversible 1-electron step, therefore, the proposed biologically active species can be selectively generated and studied. The present investigation utilizes this feature to study the interaction of the 1-electron species generated *in situ* with DNA using both electrolytic and voltammetric techniques. The two methods are not necessarily complementary, with longer time-scales and higher reactant and product concentrations being a necessity during electrolysis experiments. Together with the often short life-times of reduction products, it is not surprising that the chemical pathway can be substantially altered from that portrayed by the voltammetry.

Target specificity was investigated by the effect of the individual DNA bases and ribose at a range of concentrations on the voltammetry of the drug, in particular the stability of the 1-electron reduction product. We have previously used this approach to investigate the interaction of a number of nitroaromatic compounds with DNA and the DNA bases.[10,11]

MATERIALS AND METHODS

TPZ was obtained from Prof. J.M. Brown, Stanford University, California. Dimethylformamide (DMF) and acetonitrile (CH_3CN), spectroscopic grade, and DNA bases and ribose were purchased from the Aldrich Chemical Company. Dry DMF was stirred over KOH to remove formic acid, then distilled from CaO immediately prior to use. *Escherichia coli* DNA was purchased from the Sigma Chemical Company and further prepared as described previously.[12]

Electrochemical experiments employed a PAR 262A polarographic analyzer interfaced with a PAR 303A cell stand and a Bausch and Lomb RE 0088 x-y recorder. Dimethylformamide and CH_3CN used 0.1 M tetra-*n*-butylammonium tetrafluoroborate ($TBABF_4$) as the supporting electrolyte, and were purged with solvent-saturated O_2-free N_2 prior to measurements. The concentration of TPZ was typically 0.2 mM. Concentrations of DNA bases and ribose, varied from 0.1 to 3.0 mM and were added to the voltammetric cell as a freshly prepared DMF stock solution (0.1 M; 10 mM; 5 mM; 50 mM for T, C, A and ribose respectively) or as the pre-weighed base. DNA was added as a 1 mg/ml solution in 0.1 SSC aqueous buffer. Cyclic voltammetry (CV) used a hanging drop mercury electrode, automatically renewed after each voltammogram, at a scan rate of 200 mV/s. A platinum wire was used as the counter electrode and all potentials were measured against an aqueous Ag/AgCl reference electrode.

Controlled potential electrolytic reductions were performed at 0.1 and 1.0 mM TPZ concentrations in DMF using a mercury pool cathode and a Ag/AgCl anode at room temperature under an N_2 atmosphere. Coulometry was carried out using an integrating multivoltmeter (Time Electronics) connected across a 1 Kohm

resistance in the reduction circuit. During the course of the reduction, samples were removed for voltammetric analysis (as described above) and UV/visible spectra were recorded using a Pye Unicam SP8-150 spectrophotometer. Electrolysis was also carried out in the presence of DNA at a drug:DNA(nucleotide) ratio of 0.25 with samples being removed for viscosity measurements.

The viscosity of the DNA was measured using an Ubbelohde-type suspended capillary viscometer at 21 °C. The flow times were averages of triplicate readings to an accuracy of 0.1 s. The flow time of the electrolytic medium was also determined, and the specific viscosity (η_{sp}) calculated by the equation:

$$\eta_{sp} = (\eta - \eta_0)/\eta_0$$

where η is the flow time of the sample and η_0 that of the medium.

RESULTS

Cyclic voltammetric studies of TPZ in DMF or CH_3CN showed a complex reduction mechanism with five or three reductions respectively.[9] The first reduction step was a quasi-reversible 1-electron addition, followed by up to four subsequent reduction steps which were all highly irreversible in nature. The voltammetry was further complicated by a chemical reaction following the most negative reduction steps to give a new redox-active species, clearly detected by CV between the original first and second reduction steps. The present investigation was primarily concerned with the 1-electron addition product, the proposed biologically active species. By restricting the potential range, the first reduction step could be studied in isolation, with similar voltammetric characteristics in both DMF and CH_3CN ($E_{1/2} = -1.025$ and -1.03 V respectively). The first reduction product can be oxidized to the original material, as observed by the corresponding return wave when the potential scan direction was reversed. The stability of the reduction product was quantified by the return-to-forward peak current ratio, ip_r/ip_f. This value was strongly influenced by the quality of the solvent. In spectroscopic grade DMF, the ip_r/ip_f ratio was reproducibly measured as 0.67. (For a fully reversible couple ip_r/ip_f = unity.) The ratio was less using freshly distilled DMF, at times being unmeasurable as no return wave was in evidence. Chemical reversibility increased by the addition of 5 µl of water, although the ratio did not increase above 0.67. Further addition of water up to 50 µl resulted in increasing encroachment of the more negative reduction steps, which ultimately affect the reversibility of the first reduction step. The ip_r/ip_f value of 0.67 indicated that there was a tendency for the product to undergo further chemical reaction (an EC mechanism). The trend increased with an increase in drug concentration (as monitored by a decrease in ip_r/ip_f) in line with a second order reaction. A TPZ concentration of 0.2 mM and the voltage scan rate of 200 mV/s were chosen to minimize the following chemical reaction and to give a well-resolved return wave. Spectroscopic grade solvents were used for all interaction studies.

The interaction of reduction products with biological targets can be studied directly from the CV.[9,10] If interaction occurs between the reduction product and the biological target characteristic redox parameters of the compound will change, primarily the ip_r/ip_f ratio. Addition of 1 ml of an aqueous DNA solution to give a drug:DNA(nucleotide) ratio of 0.6 resulted in a decrease in the stability of the reduction product by 15% (Table I). An analogous experiment with metronidazole produced a decrease in the stability of the nitro radical anion by 25%. (These differences have allowed for the addition of aqueous buffer on the ip_r/ip_f values.)

The influence of the individual DNA bases and ribose on the voltammetry of TPZ has also been studied. All resulted in changes to the voltammetry, primarily to the more negative

TABLE I Influence of the DNA bases and ribose on the CV parameters of Tirazazamine at a drug target ratio of 1

Target	CV Measurements[a]			
	$E_{1/2}$ (V)	ΔEp (mV)	ip_r/ip_f	$\%\Delta ip_r/ip_f$
Control[b]	−1.025	45	0.67	
Thymine	−0.98	75	0.55	−18
Adenine	−0.985		No return wave in evidence	
Guanine	−0.995	45	0.62	−7.5
Cytosine	−1.01	35	0.817	+22
Ribose	−0.99	30	0.804	+20
DNA[c]	−0.995	60	0.57	−15

[a] All CV were recorded in DMF (0.1 M $TBABF_4$) using $E_i = -0.8$ V and $E_\lambda = -1.3$ V with a scan rate of 200 mV/s.
[b] The voltammetry of Tirapazamine only.
[c] Measured at drug/target of 0.6. The CV parameters allow for the 0.1 SSC aqueous buffer used with DNA.

reduction steps, characterized by a varying positive potential shift (from 10 to 170 mV), increased resolution of the second reduction and improved separation of the third and fourth reduction steps. Table I compares the CV parameters for the first reduction couple at a TPZ:target ratio of 1. It should also be noted that although no return wave was in evidence after adenine addition, at a TPZ:base ratio of 2, a decrease in $\Delta ip_r/ip_f$ of 9.4% was measured for adenine compared with a 14% decrease for thymine. Voltammetric changes with ribose addition do not appear to be progressive, with further increases in the ribose concentration resulting in very minor changes to the CV.

The influence of thymine, adenine and cytosine was extended to a TPZ:base ratio of 0.05 and the CV was found to change markedly. At low base concentrations the CV retained the general characteristics of TPZ already noted. As the base concentration was further increased, the voltammetric pattern became simplified. At a TPZ:cytosine ratio of 0.067 the CV showed three reduction waves. The return wave for the first reduction was still in evidence, but was no longer influenced by inclusion of the following reduction steps in the voltammetric scan. The addition of adenine or thymine to a TPZ:base ratio of 0.2 showed two irreversible waves of approximately equal current. No return wave character was observed, irrespective of switching potential. The voltammetry was unaffected by repeat cycling, and there was no evidence of coupled chemical reactions yielding redox-active species, as in the original voltammetry of TPZ. However, as the concentration of adenine, thymine or cytosine increased, an oxidation wave linked to the reduction step was seen to develop at −0.65 V. The addition of thymine on the CV of TPZ up to a TPZ:thymine ratio of 0.2 is compared in Figure 1. No further changes to the voltammetric pattern were observed with further increases in base concentration. Similar studies with G were not possible due to its sparing solubility.

Controlled potential electrolysis was used to produce large quantities of the reduced species, including preparation in the presence of target molecules, allowing analysis of subsequent biological impact. Using DMF as the solvent at a drug:DNA(nucleotide) ratio of 0.25 (TPZ, 0.2 mM) and a potential of −1.0 V, resulted in a decrease in the specific viscosity of the DNA by 12%. Under comparable conditions of drug:DNA contact but with no reduction of the TPZ, no change in the viscosity was observed.

Electrolysis was used to further characterize the first reduction product using coulometry, UV/visible spectroscopy and CV monitoring during the reduction process. A TPZ concentration of 0.1 mM was used to further minimize the chemical reaction which follows the electron transfer step. As the reduction progressed, the current was seen to decrease to background

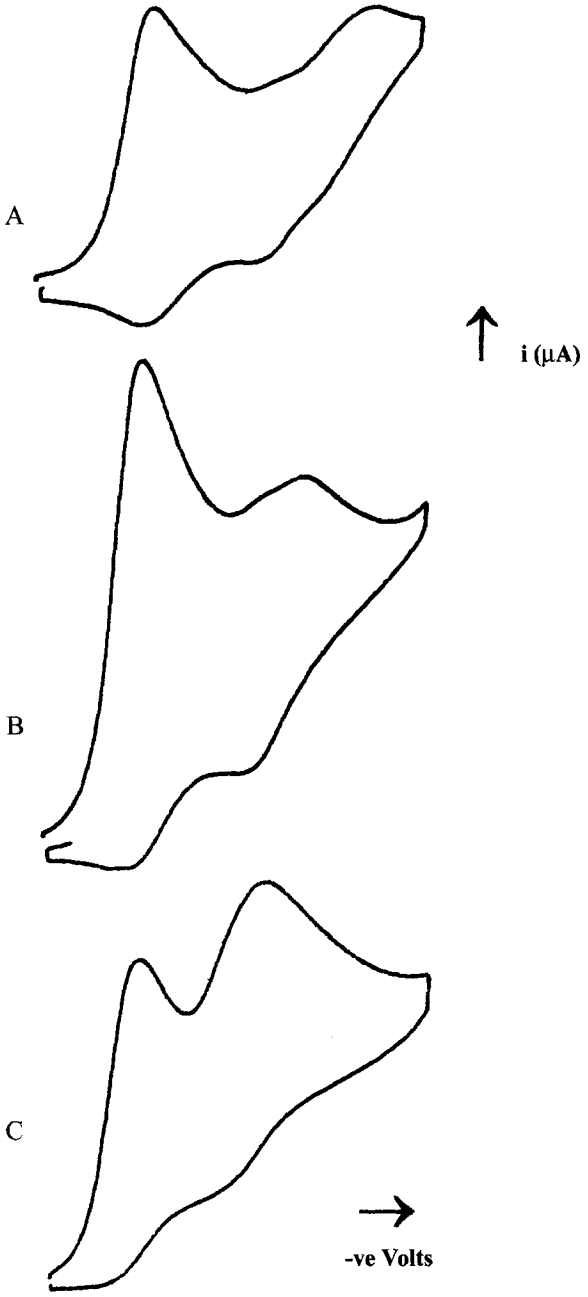

FIGURE 1 The effect of thymine addition on the voltammetry of tirapazamine in dimethylformamide ($E_i = -0.9$ and $E_\lambda = -1.6$ V; $\nu = 200$ mV/s). (A) Tirapazamine:thymine ratio of ∞; (B) Tirapazamine:thymine ratio of 1; (C) Tirapazamine:thymine ratio of 0.2.

levels. The original orange colouration of the solution changed to a distinctive bright blue. Switching the potential to −1.7 V gave a large

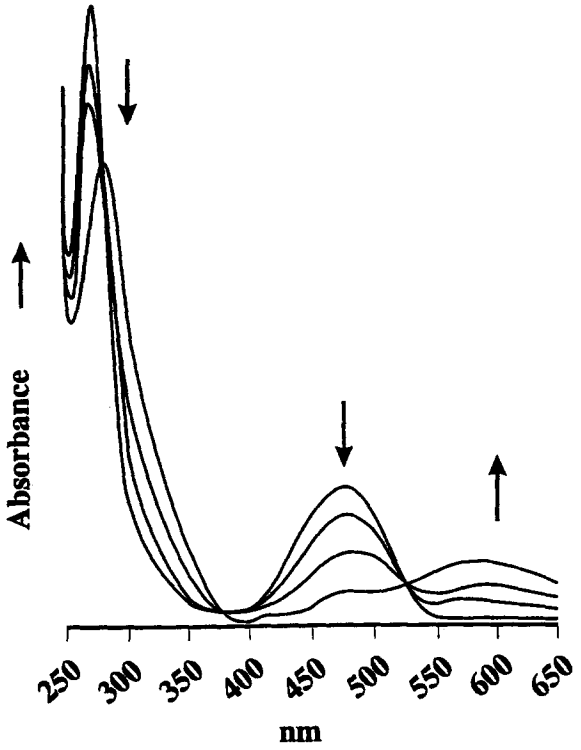

FIGURE 2 The UV/visible spectra recorded during the electrolytic generation of the first reduction product of tirapazamine in acetonitrile.

increase in current, due to the more negative reduction steps, as expected from CV observations, and a gradual decrease in current and a change in colour to a dark orange. Coulometry gave an electron requirement of $n = 3$ for the first reduction step. Comparing counts for the first step to the overall reduction gives a 1/5.44 ratio. Figure 2 shows the UV/visible spectra recorded *in situ* during the reduction of TPZ in CH_3CN. The same changes were observed in DMF, but the UV range was limited. The original band at 475 nm was seen to decrease concomitant with development of a new absorption band at 590 nm. The peak at 272 nm shifts to 270 nm, with a decrease in absorbance and a general broadening. Electrolytic oxidation or exposure to air generated the original spectrum without loss of material. No measurements were taken during electrolysis at −1.7 V, but the final reduction product(s) was not affected by O_2 exposure.

In separate, but identical experiments, samples were taken for CV analyses. Although the first reduction was still in evidence, a new chemically reversible process at −0.50 V was also observed. On air exposure there was a rapid change in colour from blue to orange with complete loss of the reversible couple at −0.50 V.

Electrolysis investigations were also undertaken at 1.0 mM TPZ, conditions which favor the following reaction. Electrolysis at −1.0 V to generate the first reduction product gave a blue/black solution, which on air exposure reverted to the original over 30–40 min. CV monitoring of an intermediate reduction sample showed an irreversible reduction corresponding to the original first reduction step of TPZ, with a new chemically reversible couple at approximately −0.50 V, formation of which was not dependent on inclusion of the TPZ first reduction step in the voltage sweep. On complete reduction, the first reduction step of TPZ was completely absent, but was regenerated (slowly) on air exposure. The reversible couple at −0.50 V was absent. CV investigations at 1.0 mM TPZ showed an irreversible first reduction step, which resulted in formation of a dark blue material in the vicinity of the working electrode and the appearance of a new chemically reversible couple at approximately −0.50 V. Repeat cycling showed that the first reduction wave of TPZ was now absent. This behaviour was not observed at routine TPZ concentrations either during normal scans or by holding the potential of the working electrode at selected values negative of the first reduction step.

DISCUSSION

Employing a limited potential range in the CV mode allowed us to selectively generate the 1-electron addition product for TPZ and study its interaction with possible biological targets.

Chemical reversibility of the TPZ first reduction step was clearly in evidence when trace amounts of water were present in the DMF solvent. This would suggest that the protonated 1-electron reduction product was responsible for the return wave observed. The unprotonated species was more likely to undergo further chemical reaction. There is substantial evidence that it is the protonated form that is responsible for biological activity of TPZ.[8,13]

Analysis of the CV in the presence of DNA showed a decrease in the stability of the first reduction product by 15%, as determined by the decrease in the ip_r/ip_f ratio. This indicated that there was less reduction product available to be oxidized back to the original material, caused by interaction with DNA. Analogous experiments using the metronidazole nitro radical anion, the proposed biologically active species, showed similar behaviour with a decrease in ip_r/ip_f of 25%.

Experiments were also performed using controlled potential electrolysis to produce large quantities of the 1-electron species in the presence of DNA. In previous experiments, the non-specific aqueous reduction of TPZ in the presence of double-stranded M13 DNA induced single and double strand breaks, as monitored by agarose gel electrophoresis, demonstrating a change from closed, covalent, circular DNA to nicked and linear forms.[14] The formation of strand breaks was also noted by a decrease in the viscosity of *E. coli* DNA. Maximum damage was achieved between a drug:DNA (nucleotide) ratio of 0.1 to 0.5.[15] The present experiments used a drug:DNA (nucleotide) ratio of 0.25 with a potential of −1.0 V to specifically generate the 1-electron reduction product. The specific viscosity of the DNA decreased by 12%. These findings by CV and electrolysis confirm that the 1-electron reduction product is the DNA damaging species.

Our CV studies were extended to examine the influence of the individual DNA components. Investigations were somewhat limited by the quasi-reversible nature of the reduction step, giving a low, initial ip_r/ip_f value. Up to a drug:target ratio of 1, adenine, guanine and thymine

resulted in interaction with the TPZ 1-electron reduction product (Table I), measured by a decrease in the ip_r/ip_f ratio in the following order of increasing activity: guanine, thymine and adenine. The metronidazole nitro radical anion showed a similar selective interaction with the DNA bases, with the greatest response found with adenine and thymine.[10] An increase in the TPZ ip_r/ip_f ratio was found upon addition of cytosine and ribose, indicating a different type of reaction to that found with thymine and adenine. Cytosine and ribose make the chemical reaction following the charge-transfer step less favourable, possibly by protonation of the first reduction product. Although the pKa of cytosine suggests that it would make an unlikely proton donor, this assignment is supported by our experimental observations on the effect of small amounts of water to the ip_r/ip_f ratio when using distilled DMF. In addition, the pKa will itself be strongly influenced by the use of the DMF aprotic solvent. The effect of ribose and cytosine may be significant for two reasons. Firstly, the stability of the reduction product should influence the biological activity of the compound. Secondly, the mechanism may be comparable to hydrogen abstraction from DNA by the TPZ reactive species leading to strand breaks.[16,17]

At higher biological target concentrations, the CV analysis is further complicated by the substantial changes produced to the voltammetry of the drug (Figure 1). The significance of these observations is under further investigation, but clearly requires a greater knowledge of the overall reduction mechanism of TPZ and related *N*-oxide compounds.

Initially the electrolysis investigations appeared to contradict the results from the CV. The electrolysis potential was chosen to selectively generate the first reduction product, with a low drug concentration to diminish the tendency to undergo a chemical reaction following the charge-transfer step and electrolysis conditions to maximize reduction rate. The reduction proceeded to completion with a fall in current to background levels and the solution changing colour from orange/yellow to bright blue. Unfortunately, the blue colouration was not due to the TPZ 1-electron addition product. CV monitoring of the electrolysis showed the expected decrease in the first reduction wave, but also showed development of a new response at mild potentials. At the potential for TPZ reduction this new species would be immediately reduced, explaining the coulometric result of $n = 3$. The slow air oxidation to quantitatively regenerate TPZ is explained by the relatively mild redox potential and the molecular rearrangements required to form the starting material as monitored by CV or spectrophotometrically (Figure 2).

The assignment was confirmed by CV studies at high TPZ concentrations. The CV showed the formation of a new redox-active species dependent on inclusion of the first reduction step in the voltage sweep, at a potential corresponding to the process observed from the electrolysis solution (but where a voltage sweep was not necessary). A blue colouration was seen to form in the vicinity of the working electrode. This behaviour was only observed by CV at high TPZ concentrations.

The difference in redox behaviour found between electrolysis and survey CV measurements reflects the longer time-scale and high concentration effects encountered when using electrochemistry to synthesize redox products. The blue colouration was due to the formation of the new (as yet unidentified) redox-active species which formed with time as the concentration of the TPZ 1-electron addition product increased.

These investigations show the advantages of using an *in situ* CV method for measuring the interaction between drug reduction product and possible biological target molecules. Greater control can be applied to the redox pathway so that a particular couple can be selected and studied in detail, without interference from

further electron transfer steps or linked chemical reactions. By applying an *in situ* analysis, we avoid the need to prepare large quantities of reactive material and make measurements under more relevant conditions. Naturally, care must be taken when interpreting data when using free DNA bases as their properties will change when incorporated into DNA, but they provide a useful reference point, and these physico-chemical observations should always be supplemented by biological assays. Work is continuing to fully assign the reduction mechanism for TPZ and other benzotriazines and to investigate the biological significance of the drug/target studies and the concentration effects observed during electrolysis experiments.

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References

[1] J. von Pawel, R. von Roemeling, U. Gatzemeier, M. Boyer, L.O. Elisson, P. Clark, D. Talbot, A. Rey, T.W. Butler, V. Hirsh, I. Olver, B. Bergman, J. Ayoub, G. Richardson, D. Dunlop, A. Arcenas, R. Vescio, J. Viallet and J. Treat (2000) Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATAPULT I study group. Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors. *Journal of Clinical Oncology*, **18**, 1351–1359.

[2] B.G. Wouters, L.H. Lang and J.M. Brown (1999) Tirapazamine: a new drug producing tumor specific enhancement of platinum-based chemotherapy in non-small-cell lung cancer. *Annals of Oncology*, **10**, Supplement 5, S29–S33.

[3] J.M. Brown (1993) SR4233 (Tirapazamine): a new anticancer drug exploiting hypoxia in solid tumors. *British Journal of Cancer*, **67**, 1163–1170.

[4] M.A. Baker, E.M. Zeman, V.K. Hirst and J.M. Brown (1988) Metabolism of SR4233 by Chinese hamster ovary cells: basis of selective toxicity. *Cancer Research*, **48**, 5947–5952.

[5] J.H. Tocher, N.S. Virk and D.I. Edwards (1990) Electrochemical studies and DNA damaging effects of the benzotriazine-*N*-oxides. *Biochemical Pharmacology*, **39**, 781–786.

[6] J.W. Evans, K. Yudoh, Y.M. Dalahoussaye and J.M. Brown (1998) Tirapazamine is metabolized to its DNA-damaging radical by intranuclear enzymes. *Cancer Research*, **58**, 2098–2101.

[7] J.T. Hwang, M.M. Greenberg and K.S. Gates (1999) Reaction of the hypoxia-selective antitumour agent tirapazamine with the C1′-radical in single-stranded and double-stranded DNA: the drug and its metabolites can serve as surrogates for molecular oxygen in radical-mediated DNA damage reactions. *Biochemistry*, **38**, 14248–14255.

[8] J.H. Tocher, N.S. Virk and D.I. Edwards (1990) DNA damaging effects and voltammetric studies of the hypoxic cell toxin 3-amino-1,2,4-benzotriazine-1,4-dioxide, SR4233, as a function of pH. *Biochemical Pharmacology*, **40**, 1405–1410.

[9] J.H. Tocher and D.I. Edwards (1994) Electrochemical studies of tirapazamine: generation of the one-electron reduction product. *Free Radical Research*, **21**, 277–283.

[10] J.H. Tocher and D.I. Edwards (1994) Evidence for the direct interaction of reduced metronidazole derivatives with DNA bases. *Biochemical Pharmacology*, **48**, 1089–1094.

[11] J.H. Tocher, D.I. Edwards and A. Thomas (1994) The reactivity of chloramphenicol reduction products with DNA bases. *International Journal of Radiation Oncology, Biology and Physics*, **29**, 307–310.

[12] D.A. Rowley, R.C. Knight, I.M. Skolimowski and D.I. Edwards (1979) The effect of nitroheterocyclic drugs on DNA: an *in vitro* model of cytotoxicity. *Biochemical Pharmacology*, **28**, 3009–3013.

[13] K. Laderoute, P. Wardman and A.M. Rauth (1988) Molecular mechanisms for the hypoxia-dependent activation of 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR4233). *Biochemical Pharmacology*, **37**, 1487–1495.

[14] R. Walmsley (1995) Effects of the benzotriazine-di-*N*-oxide tirapazamine upon DNA. PhD Thesis, University of East London.

[15] N.S. Virk (1993) DNA damaging effects of benzotriazine-*N*-oxides. PhD Thesis, University of East London.

[16] J.H. Elwell, B.G. Siim, J.W. Evans and J.M. Brown (1997) Adaptation of human tumor cell lines to tirapazamine under aerobic conditions: implications of increased antioxidant enzyme activity to mechanism of aerobic cytotoxicity. *Biochemical Pharmacology*, **54**, 249–257.

[17] A.M. Rauth, T. Melo and V. Misra (1998) Bioreductive therapies: an overview of drugs and their mechanisms of action. *International Journal of Radiation Oncology, Biology and Physics*, **42**, 755–762.