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Charge transfer-oxy radical mechanism for anti-cancer agents[1]

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Summary: The proposal is advanced that anti-cancer drugs generally function by charge transfer resulting in formation of toxic oxy radicals which destroy the neoplasm. Electrochemical studies were performed with some of the main types of agents: iminium ions (adenine iminium from alkylating species, iminium metabolite of 6-mercaptopurine, nitidine, other polynuclear iminiums) and metal complexes (Pt(II)diaquodiammine-guanosine, copper salicylaldoximes). Reduction potentials ranged from −0.4 to −1.2 V. Literature data for quinones are presented and radiation is discussed. Based on the theoretical framework, a rationale is offered for the carcinogen–anti-cancer paradox and the role of antioxidants.

More than two decades ago the oxy radical hypothesis for carcinogenesis was advanced (Brues & Guzman Barron, 1951; Holman, 1956; Harman, 1956). Shortly thereafter, the proposal was placed on a broader, more systematic foundation (Harman, 1962; Kovacic, 1959 and 1960). This approach received scant attention until fairly recent times which have witnessed ever increasing support from a variety of disciplines (Ames, 1983; Mason, 1982; Demopoulos *et al.*, 1980). In general terms, the comprehensive theory states that oxy radicals are implicated in the action of most carcinogens, arising as the end product of metabolic processes, usually via charge transfer (CT). Apparently, the highly reactive radicals subsequently attack cellular DNA, as well as other crucial constituents, resulting in transformation to the oncogenic state. Specific application has been made to alkylating agents, quinones, metal complexes, iminium ions, radiation, carbon tetrachloride, 4-nitroquinoline 1-oxide, and inert bodies (Kovacic *et al.*, in press).

The initial inklings (Holman, 1956; Warburg *et al.*, 1957) that reactive oxy species may play a role in anti-cancer action was shortly followed by a better developed, more comprehensive approach (Kovacic, 1959). A baffling paradox of oncology is the well-known phenomenon that generally the substances which induce cancer are also antineoplastic. If the premise is valid that these agents cause cancer by producing excessive amounts of oxy radicals, it may well be that their ability to combat the condition is intimately related to the same chemical property. An essential component of the overall picture is the corollary that many tumor cells are more susceptible than normal ones to elevated concentrations of oxy radicals, thus providing the requisite specificity. Supporting evidence may be found from the early days of oncology (Kovacic, 1959), as well as newer data which will be presented in the discussion section.

Recently the suggestion was made that iminium species (**1**), usually in conjugated form, play important roles biologically in a

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variety of redox transformations (Kovacic, 1984). These entities might then function catalytically at the active site as electron conduits for the formation of superoxide, a precursor of other oxy radicals (Fridovich, 1983). This concept is now applied to the anti-cancer domain.

The principal objective of the present work was to determine the electrochemical characteristics of several main categories of antineoplastic agents: iminium ions (adenine iminium from alkylating species, iminium metabolite of 6-mercaptopurine, nitidine, other polynuclear iminiums) and metal complexes (Pt(II) diaquodiammine-guanosine, copper(II) salicylaldoximes). Literature data for quinones and other CT agents are presented, and radiation is discussed. The results are treated within the context of the unifying theory for anti-cancer action involving CT with production of toxic oxy radicals. The carcinogen—anti-cancer paradox is addressed, as well as the role of antioxidants.

Materials and methods

Isoquinolinium salts **7** and **8** were obtained from Prof Mark Cushman (Cushman *et al.*, 1984). Literature methods were used for synthesis of purine-6-sulfinate **6** (mp 178°C (dec.), lit. (Doerr *et al.*, 1961); mp 175°C (dec.), 3-benzyladenine chloride **3** (mp 254–260°C with prior darkening, lit. (Abshire and Berlinquet, 1964; mp 261–267°C), 3-benzyladenine (**3**-HCl) (mp 268–270°C, with prior darkening, lit. (Abshire & Berlinquet, 1964; mp 284–287°C) and 1-methyladenosine iodide **4** (mp 190–195°C) (dec.) (Jones & Robins, 1963). Elemental analyses were satisfactory for the compounds whose melting points differed appreciably from literature values. Benzothiazoloquinolinium salts **9** (Cox *et al.*, 1982 and unpublished results), and copper(II) salicylaldoximates **11** (Lumme & Korvola, 1975; Lumme *et al.*, 1984) were prepared as described. cis- and trans-Diaquodiammine platinum(II) nitrates were obtained from the corresponding DDPs by stirring with two equivalents of $AgNO_3$ in H_2O for 3 and 1 h, respectively, and filtering the AgCl precipitate (Marcelis *et al.*, 1980). The solution was evaporated to dryness in a vacuum over H_2SO_4 to yield the product. Complex formation was attempted (Dehand & Jordanov, 1976) with guanosine and the cis-diaquo reagent for 30 min, since this Pt compound is reported to be quite reactive (Marcelis *et al.*, 1980). However, no precipitate formed; the solution was evaporated under vacuum to furnish a solid material.

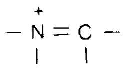
Cyclic voltammetry and polarography were performed on an ECO model 550 potentiostat with a PARC model 175 waveform generator. All solutions were degassed for 15 min with pre-purified dinitrogen that was passed through an oxygen scrubbing system. The working electrodes were a platinum flag or a hanging mercury drop (HMDE). Reference electrodes were an IBM aqueous Ag/AgCl or a Corning SCE both in saturated KCl. The counter electrode in all cases was a platinum wire. The supporting electrolyte was tetraethylammonium perchlorate (G.F. Smith Chemical Co.). The solvents, N,N-dimethylformamide and dimethyl sulfoxide were obtained from Aldrich Chemical Co. in the highest possible purity, in addition to cis-DDP, trans-DDP and guanosine hydrate. Buffer solutions of pH 3.3, 3.9 and 4.8 ($HOAc/OAc^-$) (compound **6**) and pH 6 (50% ETOH/buffer, KHP) (compounds **7** and **8**) were used for cyclic voltammetry.

Results and discussion

Iminium ions

1. Purines

(a) *Alkylated DNA models*: The alkylating agent class contains a large group of antineoplastic agents, including nitrogen mustards, epoxides, aziridines, triazenes, N-nitroso compounds, and alkyl alkanesulfonates (Reich, 1981). Some have progressed to the stage of practical use in chemotherapy. As is well established, the diverse types also



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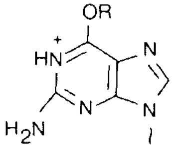

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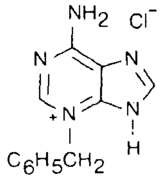

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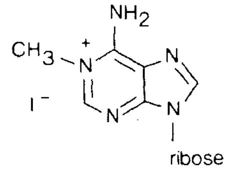

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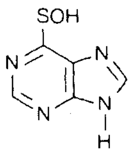

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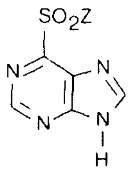
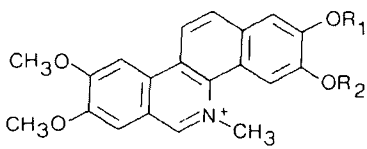


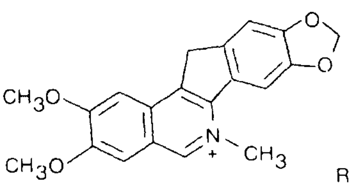
a) Z=H b) Z=Na

6



a) $R_1+R_2=CH_2$ b) $R_1=H$ $R_2=CH_3$

7



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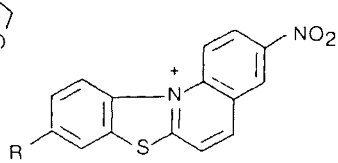
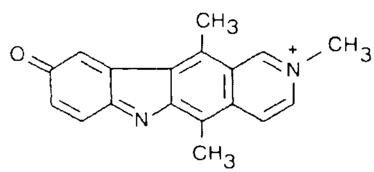


a) R=H b) $R=OCH_3$

9



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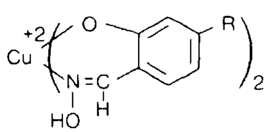
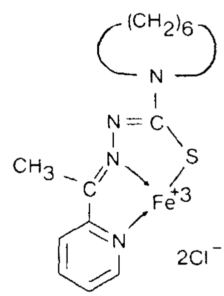


a) R=H b) R=OH

11



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generally function as carcinogens (Miller & Miller, 1983). Concomitant production of oxy radicals has been observed with various members (Ames, 1983; Floyd, 1982). Although the precise role of these reactive intermediates has not been ascertained, it appears that DNA strand cleavage may be a crucial event (Floyd, 1982).

In a recent investigation of the mechanism of carcinogenesis, a novel proposal was advanced in which the salt form (iminium) of alkylated nucleic acid was assigned a key function as a CT agent (Kovacic *et al.*, in press). The purines (guanine and adenine) of DNA are the principal targets of attack (Miller and Miller, 1983). For example, the ionic structure **2**, a conjugated form of iminium (**1**) is generated from O-6 alkylation of guanine and could conceivably undergo one-electron reduction. Electrochemical data from the literature (Dryhurst, 1977) and our own studies (Kovacic *et al.*, in press) are in reasonable accord with the current picture relating site of alkylation and defect persistence to oncogenic response. Thus, it appears quite plausible that the salt form is functioning in a catalytic manner as a generator of toxic oxy radicals.

In order to test this concept as applied to anti-cancer alkylating agents, salts derived from alkylation of adenine and adenosine were investigated electrochemically as models. 3-Benzyladenine chloride **3** gives irreversible reduction values of about −1.0 V (Table I). Upon addition of strong base the potentials become more negative and the current drops until, with excess base, there is no reduction before the background current, due to generation of the nonreducible, nonionic base via loss of HCl. Occurrence of this transformation was confirmed by electrochemical studies on the free base which gave no reduction before background. A second model consisted of the nucleoside with the base alkylated at a different site, namely, N-1. For 1-methyladenosine iodide **4**, the most

Table I Cyclic voltammetry of N-alkylated adeninium and adenosinium halides.[a]

Compound	$[OH^-]$ mM	$-E_p$ *DMF*	$-E_p$ *DMSO*
3-Benzyladenine chloride	—	0.96	1.00
	0.49	1.03	1.19
	0.99	—[b]	—[b]
3-Benzyladenine	—	—[b]	—[b]
1-Methyladenosine iodide	—	0.96	1.23
	0.49	0.96	1.23
	0.99	—[b]	—[b]

[a] 100 mV/s, tetraethylammonium perchlorate (0.1 M), substrate (0.5 mM), Pt electrode, irreversible, vs. SCE.
[b] No reduction of substrate before background reduction.

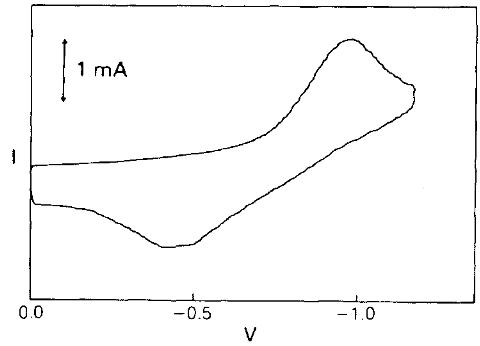

Figure 1 Cyclic voltammogram of **4** in DMF, Pt electrode, scan rate 100 mV/s.

positive figure obtained for the reduction potential was −0.96 V (DMF) (Figure 1, Table I). The results were less favorable in DMSO. The product of the reduction is probably a dimer, since coupling has been observed from electrolysis of purine bases in non-aqueous solvent (Yao *et al.*, 1976).

Our data are in agreement with previous investigations with adenine in aqueous acid. $E_{1/2}$ values, −1.05 to −1.07 V, were reported, which varied linearly with pH (Dryhurst, 1977). Adenosine and adenylic acid behaved similarly. Evidently salt formation occurs by preferential protonation at N-1 (Saenger, 1984).

There are several possible sites for adenine alkylation. The preferred one in vivo is generally the N-1 position. Reaction at N-7 is also commonly observed, whereas N-3 attack varies in degree (Shooter, 1972; Rajalakshmi *et al.*, 1982). Alkylation at any of these positions would produce a potential CT agent capable of catalytic operation. The N-3 position has been suggested as an important locale in the carcinogenic process (Lijinsky, 1976). It is significant that N-7 adenine salts possess physiological activity (Iio *et al.*, 1985).

(b) *6-Mercaptopurine:* The properties of this drug are summarized in Table VII. It, as well as related materials, is evidently converted to the corresponding nucleotide (Ishiguro *et al.*, 1984) via the nucleoside (Chabner, 1981; Christie *et al.*, 1984) followed by insertion into the DNA chain. The thiol appears to be oxidized to the unstable sulfenic acid **5** which undergoes further conversion to the isolable sulfinic acid **6a** (Hyslop & Jardine, 1981; Nelson, 1982). The acids could exist in an ionic (iminium) form (cf. **2**) either from intra- or intermolecular nuclear protonation.

Since an oxidative metabolite is thought to be the active agent, we obtained data on the reduction potential for **6**. In DMF the E_p varies from −1.0 V for the iminium from nuclear protonation to >−2.0 V for the sodium salt (**6b**) (Table II). In aqueous buffer **6** exhibits potentials (V) that vary linearly with pH ($E_p = -0.44 - 0.100$ pH); the most positive value was −0.77 V (pH 3.3). Hence reduction is facilitated by increasing acidity. The results in aqueous media are in agreement with data (V) from an earlier study (Dryhurst, 1969) in which **6** exhibited $E_{1/2} = -0.37 - 0.094$ pH (pH 1–9.1). Reduction involved the N-1=C-6 bond giving the dihydro product (Dryhurst, 1977). Also included were the parent thiol, $E_{1/2} = -0.79 - 0.116$ pH (pH 0–5), purine 6-sulfonic acid, $E_{1/2} = -0.45 - 0.078$ pH (pH 1–7), and 6-purinyldisulfide, $E_{1/2} = -0.0$ V. From these findings Dryhurst concluded that **5** should reduce at a value between those for **6b** and the disulfide.

Table II Cyclic voltammetry of purine-6-sulfinic acid.[a]

[*Acid*] mM		$-E_p$ DMF	$-E_p$ H_2O[b]
—	—	>2.0	—[c]
$HClO_4$	0.46	1.24	—[c]
$HClO_4$	0.91	1.03, 1.23	—[c]
$HClO_4$	1.3	1.00, 1.22	—[c]
HOAc	pH 3.3	—[c]	0.77
HOAc	pH 3.9	—[c]	0.83
HOAc	pH 4.8	—[c]	0.92

[a] 100 mV/s, tetraethylammonium perchlorate (0.1 M, DMF), **6b** (0.5 mM), HMDE vs. SCE, irreversible.
[b] HOAc/OAc⁻ buffer.
[c] Not examined electrochemically.

Table III Electrochemistry of fused derivatives of quinolinium and isoquinolinium salts.[a]

Compound	$-E_p$ *DMF*	$-E_p$ *DMSO*	$-E_p$ H_2O[b]	*Electrode technique*[c]
7a	0.90	—[d]	—[d]	Hg, P
	1.07	1.09	—[d]	Pt, CV
	—[d]	—[d]	1.15	Hg, CV
7b	0.99	—[d]	—[d]	Hg, P
	1.01	—[d]	0.89	Hg, CV
	1.11	1.14	—[d]	Pt, CV
8	1.24	—[d]	—[d]	Hg, P
	1.25	—[d]	1.35	Hg, CV
	1.34	1.35	—[d]	Pt, CV
9a	0.39, 1.18	0.42, 1.12	—[d]	Hg, P
	0.42, 1.19	0.45, 1.17	—[d]	Hg, CV
	0.65, 1.21	0.64, 1.16	—[d]	Pt, CV
9b	0.42, 1.18	0.45, 1.12	—[d]	Hg, P
	0.45, 1.20	0.47, 1.16	—[d]	Hg, CV
	0.66, 1.17	0.67, 1.17	—[d]	Pt, CV

[a] 100 mV/s, tetraethylammonium perchlorate (TEAP, 0.1 M), substrate (0.5 mM), vs. SCE.
[b] Buffer (KHP) pH 6, no TEAP.
[c] P—polarography, CV—cyclic voltammetry.
[d] Not examined electrochemically.

2. Fused derivatives of quinolinium and isoquinolinium salts. This class is represented by the alkaloids nitidine **7a** and fagaronine

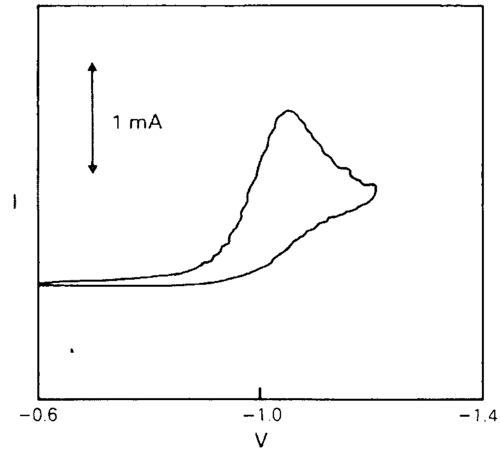

Figure 2 Cyclic voltammogram of **7a** in DMF, Pt electrode, scan rate 100 mV/s.

7b, the indenoisoquinolinium salt **8** and 3-nitrobenzothiazolo(3, 2-a)quinolinium salts **9a, b**. Studies on **7** and the analogue **8** gave values ranging from −0.90 to −1.15 V (Table III) for **7** and −1.25 to −1.35 V for **8**. The methoxyl substituent is known to result in more negative potentials (Zuman, 1967a). Cyclic voltammetry (CV) (Figure 2) gives irreversible reductions. On the other hand, calculations from polarography (P) and CV indicate reversible behavior. The $E_p - E_{p/2}$ (CV) and $E_{3/4} - E_{1/4}$(P) values of 60 mV are in reasonable agreement with the theoretical values of 57(CV) and 56(P) mV for a one-electron process. Isoquinolinium salts are known to undergo one electron reduction with formation of the 1,1′-dimer (Bradsher, 1981). The nitrobenzothiazoloquinolinium salts (**9a, b**) give multiple reduction values (Table III) (Figure 3); the most positive range from −0.39 to −0.65 V (irreversible). The more negative waves, about −1.2 V, are reversible. Calculations on the first wave provide values similar to those from **7**, namely, 63(CV) and 60(P) mV. Apparently the reductions are followed by a fast follow-up step. There are two predominant electroactive sites associated with **9**, namely, the nitro group and the iminium ion. The literature $E_{1/2}$ for nitrobenzene is −0.62 V (Wheeler, 1963). Enhancement in the positive direction in our case is due to a more extended, electrophilic system of conjugation. Substitution of methoxyl for hydrogen in the benzothiazole ring has the effect of making the reductions more negative by about 0.03 V as a result of electron donation, in agreement with the reported effect of the 4-methoxyl group in the 1-phenylpyridinium ion, i.e., $\Delta E_{1/2}$ was more negative by about 0.03 V (Zuman, 1967a).

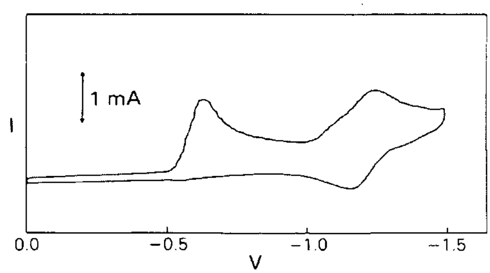

Figure 3 Cyclic voltammogram of **9a** in DMF, Pt electrode, scan rate 100 mV/s.

The activity of 7 has been correlated with the presence of the iminium site (Caolo & Stermitz, 1979), in keeping with our theoretical framework. Also N-methylphenanthridinium salts are known to undergo charge transfer (Parkanyi & Leu, 1975). It is reasonable to associate the activity of **9** in part with nitro or the nitroso reduction product, since compounds of this type are used in cancer therapy (Docampo & Moreno, 1984; Murray & Meyn, 1985). These substances (**7–9**) may exert their activity by binding to DNA (Baez *et al.*, 1983; Cushman *et al.*, 1984). Related anti-tumor alkaloids include coralyne (Cox *et al.*, 1982) and sanguinarine (Nandi & Maiti, 1985).

3. Ellipticines. Most members of this class are anti-tumor agents. Metabolites and various derivatives incorporate quinoneimine and iminium, e.g., **10**. The results from extensive studies (Paoletti *et al.*, 1983) are summarized in Table VII. Electrochemical data demonstrate the ability of the hydroxylated metabolite to function as a charge transfer entity (Paoletti *et al.*, 1983).

Recent reviews deal with iminium ions in the alkaloid category (Knabe, 1979) and from oxidative metabolism of xenobiotics (Overton *et al.*, 1985). The iminium charge transfer theory appears broadly applicable to a wide variety of biologically active agents (Kovacic, 1984), carcinogens (Kovacic *et al.*, in press), drugs (quinoxaline-di-N-oxides) (Ryan *et al.*, 1985), MPTP (Ames *et al.*, in press a), phencyclidine, nicotine and spermine metabolites (Ames *et al.*, in press b), antimalarials (Ames *et al.*, 1985c), mesoionic betaines (Ames *et al.*, 1986d) and benzodiazepines (Crawford *et al.*, in press).

Metal complexes

Metal species are known to elicit a variety of physiological responses. Specific chemical reactions that have been observed include oxygen radical formation (Ames, 1983; Stern, 1985) and DNA strand cleavage (Furst

& Radding, 1984) (Table VII). Formation of complexes with DNA is reported for some cases (Furst & Radding, 1984; Saenger, 1984) (Table VII).

1. cis-DDP. The most prominent member of the anti-cancer group is cis-DDP. Several reviews summarize much of the work (Roberts & Thomson, 1979; Rosenberg, 1980; Barton & Lippard, 1980). Binding of Pt(II) to guanine of DNA is known to occur, and is believed to have marked biochemical and pharmacological significance (Pinto & Lippard, 1985; Macquet & Theophanides, 1975; Ciccarelli *et al.*, 1985). Considerable effort has been devoted to structural analysis of the DNA-Pt(II) complex (Sherman *et al.*, 1985; Marcelis *et al.*, 1980; Rosenberg, 1980).

Since there is apparent conversion to the diaquodiammine metabolite *in vivo* (Carsey & Boudreaux, 1980), attention was centered on this form in the electroreduction studies. Guanosine was used as the model ligand. Cyclic voltammetry data for the Pt complexes are presented in Table IV. All reductions are irreversible. No reduction occurs before background for cis-DDP and guanosine. Experiments with the cis-diaquodiammine Pt(II)-guanosine complex (solid or in solution) revealed approximately the same E_p values (−0.96 to −1.0 V) as for the Pt(II) precursor. The similar results may be due to involvement of supporting electrolyte since added salt is known to alter the 1:1 $Pt(II)(H_2O)_2(NH_3)_2$-guanoside complex in solution (Marcelis *et al.*, 1980). No reduction occurs before background for trans-DDP, and the corresponding diaquo derivative is reduced at −1.20 V. The trans-diaquodiammine Pt(II)-guanosine complex (1:1 and 1:2 in solution) gave E_p values that are more negative (−1.3 to −1.6 V). Thus, the reduction potentials in the cis series are more positive than for the trans counterparts. Since trans-DDP is less active (Cleare, 1974) than the cis-isomer a correlation exists between potency and ease of electroreduction, in accord with the general mechanistic theme. Prior rationale for the difference in activity has been summarized (Johnson *et al.*, 1985).

Table IV Cyclic voltammetry of Pt(II)-guanosine complexes.[a]

Compound	$-E_p(V)$
cis-DDP	NR[b,c]
trans-DDP	NR[b,c]
Guanosine	NR[b,c]
cis-$Pt(II)(H_2O)_2(NH_3)_2$	0.96
cis-$Pt(II)(H_2O)_2(NH_3)_2$-guanosine	0.96
cis-$Pt(II)(H_2O)_2(NH_3)_2$-guanosine (1:1 solution)	1.0
trans-$Pt(II)(H_2O)_2(NH_3)_2$	1.20
trans-$Pt(II)(H_2O)_2(NH_3)_2$-guanosine	
(1:1 solution)	1.45
(1:2 solution)	1.30, 1.60

[a] Pt flag, tetraethylammonium perchlorate (0.1 M), substrate (0.5 mM), *vs.* Ag/AgCl, 100 mV/s.
[b] No reduction.
[c] 200 mV/s.

The proposed pathway entailing catalytic production of oxy radicals is consistent with effectiveness of the Pt drug at low doses (Rosenberg, 1980; Barton & Lippard, 1980). The toxicity is reduced by mercapto-containing compounds that are well known antioxidants (Nagy *et al.*, 1986; Kempf *et al.*, 1986). Other radical scavengers such as α-tocopherol and N,N′-diphenyl-*p*-phenylenediamine exerted a similar effect (Sugihara & Gemba, 1986). The investigators proposed free radical damage by the drug. Also thiols protected against mutagenesis (Nagy *et al.*, 1986) a condition generally attributed to oxy radicals (Kovacic, 1984). There is evidence for a close relationship between mutagenesis and carcinogenesis (Slaga, 1983). Chromosomal aberrations, primarily chromatid breaks, are known to be induced by cis-DDP (Flessel *et al.*, 1980).

2. Complexes of copper and iron

(a) *Copper*: Some copper complexes in this category **11** incorporate salicylaldoximes as chelating agents (Lumme *et al.*, 1984). Reduction potentials for **11a** and **11b** ranged from −0.86 to −0.96 V for the most positive values with Pt as the working electrode, and from −0.71 to −0.86 V with Hg. All of the reductions were irreversible (Table V) (Figure 4).

Table V Cyclic voltammetry of copper(II) salicylaldoxime complexes.[a]

	$-E_p$		
Compound	*DMF*	*DMSO*	*Electrode*
11a	0.86, 1.29	0.93, 1.34	Pt
	0.75, 1.23	0.71, 1.18	Hg
11b	0.91, 1.26	0.97, 1.38	Pt
	0.86[b]	0.84[b], 1.28	Hg

[a] 100 mV/s, tetraethylammonium perchlorate (0.1 M), substrate (0.5 mM), irreversible, vs. SCE.
[b] Reduction with adsorption.

The difference in reduction potential for **11a** and **11b** (−0.04 to −0.05 V, Pt electrode) is in agreement with the reported effect of the hydroxyl group in anthrone, i.e., $\Delta E_{1/2}$ was more negative by 0.01 to 0.05 V (Zuman, 1967b). There has been a prior suggestion that electron transfer may play a mechanistic role *in vivo* (Lumme & Elo, 1985). For the related Cu(II)(3,5-diisopropylsalicylate)$_2$, evidence was provided to support the contention that hydrogen peroxide is partly involved in the anti-tumor action (Oberley *et al.*, 1983).

Another class of copper(II) coordination compounds, the thiosemicarbazones, is known to possess anticancer activity (Petering, 1980; Scovill *et al.*, 1982). Reduction of the complex derived from 2-acetylpyridine thiosemicarbazone occurs reversibly at about −0.5 V (Ames *et al.*, 1985c). The

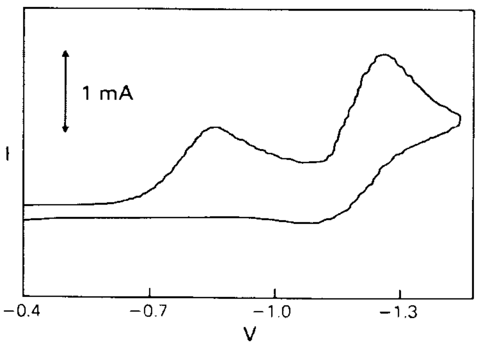

Figure 4 Cyclic voltammogram of **11a**, in DMF, Pt electrode, scan rate 100 mV/s.

related bis(thiosemicarbazone) complexes display $E_{1/2}$ values of −0.34 to −0.53 V, adjusted to SCE that are attributed to the reduction of Cu(II) to Cu(I) (Winkelmann *et al.*, 1974). According to our guiding theme, there is CT resulting in toxic oxy radicals via superoxide. Experimental support is provided by the observation that Cu(I)bis(thiosemicarbazone) is autoxidizable by oxygen (Petering, 1972), a process expected to produce superoxide.

It is relevant that interaction of heterocyclic carboxaldehyde thiosemicarbazones with DNA was observed to result in single strand cleavage (Tsiftsoglou *et al.*, 1975); preliminary association of the drug with metal may well occur. DNA scission is commonly associated with oxy radical formation (Demopoulos *et al.*, 1980). Agrawal & Sartorelli (1978) proposed that the action on DNA is of major significance for cytotoxicity.

(b) *Iron*: Iron complexes of thiosemicarbazones show antineoplastic activity (Scovill *et al.*, 1982). Compound **12** exhibits a reduction wave at −0.23 V (reversible) (Ames *et al.*, 1985c).

Proposals have been made that several well-known agents function after initial coordination with metal ion. The action of bleomycin is summarized in Table VII (Halliwell & Gutteridge, 1985a). According to current thinking (Hecht, 1979; Lown, 1982), the drug sequesters Fe(III) in the cell nucleus and intercalates or binds to DNA. Redox reactions involving the iron and oxygen take place. The reduction potential for the Fe(III) complex is −0.11 V adjusted to SCE (Melnyk *et al.*, 1981).

Adriamycin is known to be a chelating agent for a number of metal ions including Fe(II), Fe(III) and Cu(II) (Halliwell & Gutteridge, 1985a). The iron complexes bind to DNA (Gianni *et al.*, 1985) and reduce molecular oxygen to reactive radicals. DNA cleavage is observed.

3. Others. Various other metals, e.g. Rh, Ru, Sn, Ti, V and Mo, in derivative form exhibit anti-cancer activity (Cleare, 1974; Cleare & Hydes, 1980; Sadler, 1982). However, compared to cis-DDP, they have received relatively little attention. In addition, several agents, such as, α,α'-dipyridyl (Hellman *et al.*, 1983) and picolinic acid (Leuthauser *et al.*, 1982), which are effective against neoplasms, may fit into this mechanistic category based on their ability to bind metals strongly.

Quinones and iminoquinones

Quinone antibiotics have found widespread application in recent years in the treatment of malignancy (Mason, 1982; Lown, 1982; 1983; Waring, 1981). Results from extensive studies, which principally involved anthracyclines, mitomycins, streptonigrin, and saframycins, are summarized in Table VII. The toxicity, found to be oxygen dependent (Halliwell & Gutteridge, 1985a), apparently results from redox cycling of the quinone. Initial metabolic reduction to the semiquinone intermediate, which can bind to DNA (Sinha & Chignell, 1979) evidently is an essential step (Lown, 1982; Emanuel *et al.*, 1984). The overall process has been designated 'site-specific free-radical' generation (Bachur *et al.*, 1982). Inhibition of the rate of DNA scission was observed with added catalase, superoxide dismutase and free radical scavengers (Lown, 1982). However, adriamycin bound to DNA is unable to participate in redox reactions (Youngman *et al.*, 1984). Strand scission can occur in the absence of binding.

Iminoquinones have not been as extensively studied. Representative members are 5-iminodaunorubicin (Lown *et al.*, 1982), anthrapyrazoles (Fry *et al.*, 1985), and actinomycin D (Halliwell & Gutteridge, 1985a; Doroshow, 1983). Relevant characteristics are intercalation, oxy radical formation, DNA cleavage, and oxygen dependency. As in the quinone case, charge transfer has not been observed after intercalation (Emanuel *et al.*, 1984; Sengupta *et al.*, 1985). Evidence shows that redox cycling and radical generation are less facile with the imine analogues (Lown *et al.*, 1979). Several other anti-cancer agents, e.g., rhodamine 123 (Lampidis *et al.*, 1983), and an oxidative metabolite of ellipticine (Paoletti *et al.*, 1983), possess similar structures.

Table VI contains the reduction potentials for a number of substances in this general category. The $E_{1/2}$ values fall in the range, -0.20 to -1.09 V. A study revealed that the anti-tumor activity of 75% of the investigated iminobenzoquinones could be correctly classified based only on their reduction potentials (Hodnett *et al.*, 1978). Also, the iminoquinones, which exhibit more negative reduction potentials than the quinones, were found to induce less DNA strand cleavage (Lown *et al.*, 1982). The end product of anthrapyrazole reduction is the corresponding dihydro form (Showalter *et al.*, 1986).

Table VI Reduction potentials for some physiologically active quinones and iminoquinones.

Compound	*Reduction potential (V)*	*Reference*
Daunorubicin	−0.62	Rao *et al.*, 1978
Adriamycin	−0.62	Rao *et al.*, 1978
Mitomycin B	−0.20	Rao *et al.*, 1977a
Mitomycin C	−0.37	Rao *et al.*, 1977b
5-Imino-daunorubicin	−0.70	Lown *et al.*, 1982
Anthrapyrazoles	−0.98 to −1.09	Showalter *et al.*, 1986
Actinomycin D	−0.82	Nakazawa *et al.*, 1985

Table VII Characteristics of anti-cancer agents.[a]

Agent	*Generation of reactive oxygen species*	*DNA binding*[b]	*DNA cleavage*
Quinones	+	+	+
Metals	+	+	+
Bleomycin	+	+	+
Ionizing radiation	+		+
6-Mercaptopurine		+[c]	+
Alkylating agents	+	+	+
Ellipticine[d]	+	+	+

[a] See the discussion for references.
[b] Intercalation or covalent.
[c] Nucleotide insertion.
[d] And derivatives.

The exact state in which these compounds generate oxy radicals at the active site is not established with certainty. Alternatively, the ultimate agent may be a metal complex (*vide supra*).

Radiation

Relevant biological effects are summarized in Table VII (Harman, 1962; Henriksen *et al.*, 1976; Greenstock & Whitehouse, 1984). It is conceivable that indirect generation of oxy radicals also occurs. The nucleic acid bases are considerably more sensitive than the phosphate backbone to radiation (Greenstock & Whitehouse, 1984). Purines are known to form N-oxy species readily on exposure to peroxide (Robins, 1967), which might then serve as CT precursors for radicals (Kovacic *et al.*, in press). For example, adenine 1-oxide displays an $E_{1/2}$ of -0.81 V, pH 1 (Dryhurst, 1977). Also, some forms of ionizing radiation apparently give rise to cationic species that alkylate cellular constituents (Seifter, *et al.*, 1984).

Correlation of reduction potential with physiological activity is not new. Examples include anti-cancer agents (Murray & Meyn, 1985) and other categories (Hodnett *et al.*, 1978; Bogatskii *et al.*, 1971).

There are indications from prior reports that reduction potential *in vivo* may well be more favorable than *in vitro* (Kaye & Stonehill, 1952; Neta *et al.*, 1985), Both dioxidine ($E_{1/2} = -1.06$ V) (Ryan *et al.*, 1985) and 1-methyl-4-phenylpyridinium ion (cyperquat, MPTP metabolite) $E_{1/2} = -1.09$ V) (Ames *et al.*, in press, a) which display rather negative values are reported to function by oxy radical generation via CT (Ryan *et al.*, 1985; (MPTP) Markey *et al.*, 1985). Reversibility is more likely *in vivo* due to immobilization of the CT agent at the active site.

Other considerations

1. *Role of oxygen.* In our prior discussion, much evidence has been cited for the formation and involvement of activated oxygen species. It is generally believed that superoxide serves as a precursor. Support for this standpoint is provided by investigations on the beneficial influence of oxygen on drug and radiation effectiveness against cancer cells (Cadenas, 1985; Teicher *et al.*, 1981; Gupta & Krishan, 1982). The conclusion was drawn that a common mechanistic pathway pertains for the diverse agents (Gupta & Krishan, 1982; Scheulen & Kappus, 1984) in accord with the present thesis. Drug activity observed during hypoxia (Teicher *et al.*, 1981) can be rationalized by reductive stress involving radical processes (Jones, 1985).

Free radicals derived from oxygen are increasingly implicated in the initiation and progression of various diseases, and in the toxic action of numerous drugs and chemicals (Nelson, 1982; Holtzman, 1982; Sies, 1985; Halliwell & Gutteridge, 1985). The following statement also reflects a unified approach: 'Several of the chemotherapeutic agents are thought to have both their therapeutic and toxic effects by causing an oxidative stress' (Holtzman, 1982). The natural phagocytic response to foreign bodies entails attack by activated oxygen entities (Baehner *et al.*, 1982).

2. Crucial differences between malignant and normal cells. As pointed out in the introduction, an important feature of the carcinogen-anti-cancer theory is the cancer-cell property of enhanced susceptibility to reactive oxygen-containing entities. This postulate, advanced quite some time ago, was based primarily on decreased levels of catalase. Since then, other enzymes which destroy these oxy species have been discovered and investigated (Willson, 1983). The superoxide dismutase (SOD) enzyme decomposes superoxide which is generated by aerobic metabolic reactions. Presumably, protection is thereby provided from the adverse effects of oxy radicals, such as hydroxyl, which can arise from the radical anion. In fact, various reports reveal inhibition of radiation carcinogenesis by SOD (Hall & Borek, 1983). A considerable number of studies have found decreased levels of SOD in malignant neoplastic tissues (Oberley & Buettner, 1979). Mn SOD was lower in all cases *vs.* normal cells. The Cu–Zn SOD levels were diminished in many, but not all, tumors. Glutathione peroxidase has also been the object of attention. The basic premise (Kovacic, 1959) advanced more than 26 years ago has been confirmed (Alexander, 1983) and restated after the discovery of the protective role of SOD: 'If equal amounts of superoxide can be delivered to both cancer cells and normal cells, then the cancer cell should be preferentially killed because it has lower Mn SOD activity. Indeed, there is evidence that many of the existing cancer treatments actually are using this rationale because many of the anti-cancer drugs have been shown to produce superoxide' (Oberley & Buettner, 1979). However, other investigators have failed to observe any obvious relationship between resistance to ionizing radiation or radical-producing drugs and tumor cell content of the following enzymes: Cu–Zn SOD, Mn SOD, catalase, and glutathione peroxidase (Marklund *et al.*, 1982). These findings of large variations in the effectiveness of protective systems may partly account for the observed differences in response by cancer patients.

Another feature of importance is the rate of production of superoxide by tumors. If the generation is similar to or greater than the case of normal cells, then the lowered levels of protective enzymes in the neoplasms would result in enhanced sensitivity to the additional oxidative stress. Investigators have shown that tumor cell mitochondria do produce superoxide (Oberley & Buettner, 1979). In one case, the rate of formation was nearly the same as for normal tissue, whereas in another report there was a five-fold increase.

Although chemotherapy and ionizing radiation have proved beneficial in the treatment of cancer, relapse and limited applicability are commonly seen (Rosenberg, 1980). There are a number of possible rationalizations (Kovacic, 1959). In the context of the theoretical interpretation, increased concentrations of oxy radicals may not be completely effective due to the survival of a small fraction of resistant cancer cells which then proliferate. This is reminiscent of the scenario which has been encountered repeatedly with drugs, insecticides, and herbicides. Furthermore, a fine balance would pertain since the radicals which are generated to combat malignancy are also capable of inducing the same condition. Several recent studies are in harmony with the dual role concept. For instance, the incidence of second cancers in an individual was increased after treatment of the primary ones with anti-cancer drugs (Huang *et al.*, 1983). The induction of new neoplasms was observed as a delayed effect (Harris, 1979). By the same token, initiation of cancer should entail a certain degree of simultaneous inhibition. In fact, early investigators have reported precisely this type of refractory condition on application of carcinogens (Kovacic, 1959).

3. Alternate mechanisms. Although the oxy radical theory possesses many attractive features, clearly it presents an over-simplified picture of a complex phenomenon. A number of investigations reveal the important involvement of other factors, principally immunological reactions, inhibition of DNA synthesis, antimetabolite action, and DNA defect repair (Rosenberg, 1980; Roberts & Thomson, 1979; Halliwell & Gutteridge, 1985a; Lumme *et al.*, 1984; Paoletti *et al.*, 1983; Cushman *et al.*, 1984; Baez *et al.*, 1983; Doerr *et al.*, 1961; Remy *et al.*, 1984; Ciccarelli *et al.*, 1985). Specific examples of compounds that are generally believed to operate by other routes are methotrexate (antifolate) (Cole, 1970), α-difluoromethylornithine (DFMO, ornithine decarboxylase inhibitor) (Metcalf *et al.*, 1978) and 5-fluorouracil (antipyrimidine) (Cole, 1970). It is noteworthy that evidence suggests the possibility of CT in some cases. For instance, conjugated iminium species derived from pyridoxal phosphate have been designated as intermediates in the reaction of DFMO with the enzyme (Metcalf *et al.*, 1978). From X-ray data on the binary complex, N-1 protonation of the pteridine portion of methotrexate to iminium is invoked (Bolin *et al.*, 1982). From a study of the ternary complex, the drug and NADPH were shown to be in close proximity (Matthews *et al.* 1978). NADPH might be oxidized by various routes including radical or CT mechanisms (Filman *et al.*, 1982). A metal complex may also participate (Kovacic, 1984) in the case of the pyridoxal imine from DFMO. It is conceivable that several mechanisms operate in concert for certain agents. A recent unifying approach for antineoplastic agents entailed modification of DNA (Hemminki and Ludlum, 1984).

4. Other biological activity. In addition to the anti-tumor property, the various agents can display other physiological activities; carcinogenic, mutagenic, cytotoxic, and teratogenic (Magee, 1982; Johnson *et al.*, 1980; Miller & Miller, 1983; Furst & Radding, 1984; Fry, 1983). There is a relationship between antineoplastic activity and the ability to function as drugs in other areas (Kinnamon *et al.*, 1980). Perhaps some of these responses are also due to oxy radical formation via CT.

5. Role of antioxidants. In prior sections, the approach entailed treatment of an established tumor. Alternatively, the problem can be attacked via prevention of initiation by decreasing the concentration of oxy intermediates. Anti-cancer agents in this category, which act as inhibitors of carcinogenesis, would generally be labeled as antioxidants (Demopoulos *et al.*, 1980; Ts'o *et al.*, 1977). A good deal of the work has involved phenolic types, such as butylated hydroxyanisole, selenium compounds, vitamin E, vitamin C, and ethoxyquin. These substances are expected to be ineffective against existing neoplasms, and would act only to inhibit the formation of additional ones from normal cells.

Here again, it is essential to bear in mind the element of specificity. To be effective the antioxidant must reach the site at which the harmful radicals are being generated. Various characteristics of the protective agent would come to bear, including hydrophobic and hydrophilic properies. Hence, it is not surprising that many studies reveal beneficial effects of antioxidants, whereas others (Willet *et al.*, 1984) do not.

In conclusion, the theoretical scheme entails several features common to most anti-cancer agents:

1. Binding to DNA by alkylation, complexation (minor groove), intercalation, or incorporation within the chain as a special purine.
2. Presence of a charge transfer entity in the form of an iminium salt, metal complex, quinone, $ArNO_2$ or ArNO.
3. Formation of toxic oxy radicals via superoxide generated by electron transfer.
4. Attack of vital cellular constituents by oxy radicals resulting in death of the cancer.

The carcinogen—anti-cancer paradox is rationalized on the basis of similar mechanisms operating in both cases; many tumor cells are more susceptible than normal ones to the toxic effects of oxy radicals. Antioxidants appear to function by destroying harmful oxy species.

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