Free Rad. Res. Comms., Vol. 3, No. 6, pp. 347–356
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Printed in Great Britain

CHARGE TRANSFER-OXY RADICAL MECHANISM FOR ANTICANCER AGENTS: mAMSA DERIVATIVES, RHODAMINE 123, AND NICKEL SALICYLALDOXIMATE

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(Received August 25th 1986)

The proposal is advanced that many anticancer agents may function via redox reactions resulting in generation of toxic oxy radicals which destroy neoplastic cells. Cyclic voltammetry was performed with some of the main types: iminium ions (protonated mAMSA derivatives), quinone derivatives (rhodamine 123) and metal complexes (nickel(II) salicylaldoximate). In addition, relevant literature data are provided. A rationale is offered that relates electrochemical data to physiological activity.

KEY WORDS: Electroreduction, mAMSA, rhodamine 123, nickel(II) salicylaldoximate, charge transfer, anticancer, oxy radicals.

INTRODUCTION

Although the mechanisms by which xenobiotics operate are becoming better understood, many questions still remain. The carcinogen class is but one example. Several decades ago, the hypothesis was advanced that radical metabolites, e.g., oxy entities, play an important role in the cancer process.[1–6] These species can attack cellular constituents, e.g., DNA, resulting in transformations that may give rise to the oncogenic state. At about the same time oxy radicals were proposed to play a major, widespread role in anticancer action.[2,4] This concept has been supported in more recent times by various workers.[7–10] Many carcinogens display anticancer action and *vice versa*.[8] Since oxy radicals seem to be implicated in carcinogenesis, then these same entities may be involved in combating the condition. Indeed, many tumor cells are more sensitive than normal ones to elevated concentrations of oxy radicals.[4,9]

The basic scheme entails abstraction of electrons from cellular material, such as DNA or protein, by a catalytic charge transfer (CT) agent bound to the active site. Conveyance of electrons to oxygen forms toxic oxy radicals which then attack vital cellular constituents. The principal categories of CT agents are quinones, metal complexes, $ArNO_2$, and iminium moieties (**1**). In some cases, there is interference with

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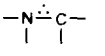
normal electrical processes, rather than superoxide formation. Application of the general theme has been made to anticancer drugs,[8] carcinogens,[11] CNS drugs (benzodiazepines),[12] antibacterial agents,[13] antimalarials,[14] mesoionics,[15] 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),[16] nicotine,[17] spermine,[17] and phencyclidine.[17] Redox cycling involving charge transfer has been gaining increased support in the mechanisms of various agents and has been reviewed.[18–20] In the case of **1**, the radical **2** would participate in the cycling.

1



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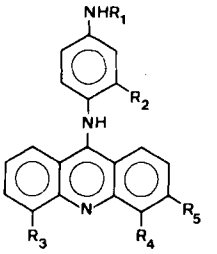
The objective of the present study was to determine the electrochemical characteristics of various drugs belonging to several main categories of anticancer agents: iminium ions (from 9-anilinoacridines), metal complexes (nickel(II) salicylaldoximate), and quinone derivatives (rhodamine 123). Related drugs are discussed. The results are treated within the context of the unifying theory for anticancer action.

MATERIALS AND METHODS

The 9-aminoacridines were obtained from Dr. Ven Narayanan (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute) (**3a–f**) and Dr. William A. Denny (Cancer Research Laboratory, University of Auckland, School of Medicine, Auckland, New Zealand) (**3g, 4**), and rhodamine 123 (**5**) was purchased from Aldrich Chemical Co. The nickel(II) salicylaldoximate complex (**6**) was prepared as described.[21] The electrolyte was tetraethylammonium perchlorate



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a. R_1 = SO_2CH_3, R_2 = CH_3O, R_3 = R_4 = R_5 = H

b. R_1 = SO_2CH_3, R_2 = CH_3O, R_3 = CH_3, R_4 = R_5 = H · CH_3SO_3H

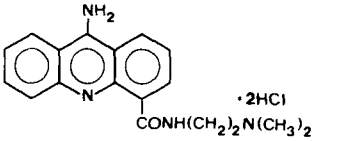
c. R_1 = $SO_2C_2H_5$, R_2 = CH_3O, R_3 = R_4 = R_5 = H, · CH_3SO_3H

d. R_1 = R_3 = R_4 = R_5 = H, R_2 = CH_3O

e. R_1 = SO_2CH_3, R_2 = R_4 = H, R_3 = CH_3, R_5 = NO_2, · HCl

f. R_1 = SO_2CH_3, R_2 = CH_3O, R_3 = CH_3O, R_4 = H, R_5 = Cl, · CH_3SO_3H

g. R_1 = SO_2CH_3, R_2 = CH_3O, R_3 = CH_3, R_4 = $CONHCH_3$, R_5 = H, · CH_3SO_3H



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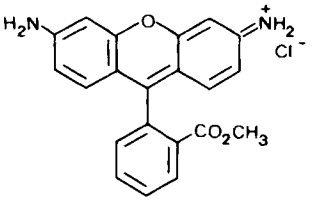

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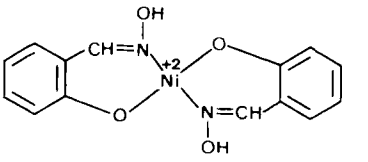

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(TEAP) (G.F. Smith Chemical Co.) and the solvents, dimethylformamide (DMF) and dimethylsulfoxide (DMSO), were obtained in the highest available purity (Aldrich Chemical Co.). Cyclic voltammetric data were recorded with a Princeton Applied Research Corp. model 174A polarographic analyzer connected to a Hewlett Packard model 7035B X-Y recorder. Solutions were deaerated for 15 minutes with prepurified nitrogen which was passed through an oxygen-scrubbing system. The electrodes consisted of either an IBM platinum flag (Pt) or hanging mercury drop (HMDE) working electrode, a saturated calomel reference (Corning), and a platinum wire as the counter. All potentials from our work and the literature are adjusted vs. the normal hydrogen electrode (NHE). The following equation was used in some of the calculations: $E_{1/2} = (E_{pc} + E_{pa})/2$. Scan rates ranged from 20 to 200 mV/s. All test solutions were prepared from substrate (0.5 mM) and tetraethylammonium perchlorate (0.1 M).

RESULTS AND DISCUSSION

Iminium Ions (mAMSA Derivatives)

Various 9-anilinoacridines are known to be broad-spectrum antitumor agents.[22,23] mAMSA (**3a**) has progressed to phase II and III clinical trials, and is effective against human acute leukemia, lymphomas, and carcinoma of the breast.[24]

The results of cyclic voltammetry (CV) at an HMDE for the 9-anilinoacridines (**3a–g**) and the 9-aminoacridine (**4**) in DMF are presented in Table I. Salts derived from $MeSO_3H$ exhibited strong adsorption. All compounds reduced irreversibly with the exception of the nitro derivative (**3e**). Free bases were obtained by neutralization of the salts with caustic. The free base mAMSA (**3a**) displayed several reduction waves with a peak potential (E_p) of -1.36 V for the first. Addition of acetic acid (0.0240 M) caused reduction to occur at a more positive value, -0.74 V. Similar behavior was exhibited by **3b–d, 3f–g**, and **4**. However, **4** reduced at much more negative values. Derivative **3e**, containing two electroactive sites (iminium and nitro) reduced quasi-reversibly with $E_{1/2}$ values in the range of -0.69 to -0.71 V for both the protonated and free-base forms. Calculated i_{pa}/i_{pc} values deviated from unity, indicative of kinetic complications,[25a] and $|E_p - E_{p/2}|$ values were dependent upon scan rate.

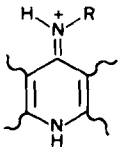
The amino group enhances basicity appreciably due to charge delocalization in the salt as shown (**7**).[14] Protonation produces a very favorable influence on ease of reduction of these acridine derivatives. Significantly, salt formation has been associated with maximum activity for mAMSA and is attributed to facilitation of site binding.[26] Likewise, various reports indicate that the related antimalarial 9-aminoa-

TABLE I
Cyclic voltammetry of mAMSA derivatives in DMF[a]

Compound	[NaOH] (mM)	[HOAc] (M)	$-E_p$ (V)
3a (mAMSA)	—	—	1.36
	—	0.024	0.74
3b	0.5[b,c]	—	1.40
	0.5	0.024	0.75
3c	0.5[b,c]	—	1.37
	0.5	0.024	0.75
3d	—	—	1.36
	—	0.024	0.79
3e	—[d]	—	0.69[e]
	0.5	—	0.71[e]
3f	0.5[b,c]	—	1.31
	0.5	0.024	0.73
3g	0.5[b,c]	—	1.23
	0.5	0.024	0.67
4	—[f]	—	1.43
	1.0	—	NR[g]
	1.0	0.024	0.99

[a] Substrate (0.5 mM), TEAP, (0.1 M), Pt vs. NHE, 100 mV/s.
[b] Acid present as methanesulfonic acid salt.
[c] CV of salt exhibits strong adsorption.
[d] Acid present as hydrochloride salt.
[e] Quasi-reversible, $E_{1/2}$.
[f] Acid present as dihydrochloride salt.
[g] No reduction.

cridines act in the iminium form.[14,27] The sidechain nitrogen exerts an unfavorable influence on reduction potential which is attributed to resonance interaction (**7**). This is demonstrated by comparison of the $E_{1/2}$ values of acridine (-0.16 V) and 9-aminoacridine (-0.83 V) at pH 5.5.[28] However, factors operating at the active site could have a favorable influence on the energetics of reduction *in vivo*, i.e., steric inhibition of resonance[14] due to site binding. Compared to the 9-aminoacridines, the 9-anilino group in mAMSA analogues should enhance reduction due in part to electron withdrawal by the phenyl moiety. The aromatic nucleus generally facilitates reduction.[29] This effect may be seen by comparison of E_p values of **4** and **3a**.



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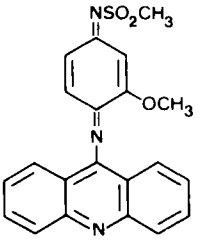
An hypothetical mechanism of anticancer action for these acridine derivatives involves CT by iminium ions at the active site with formation of toxic oxygen species that destroy cancerous tissue,[8] analogous to prior proposals for quinones and quinoneimines.[9,30,31] Pulse radiolysis of mAMSA at pH 7 has shown that the radical formed by one-electron reduction of the protonated parent is readily oxidized by several entities, including oxygen.[32] Of significance to mAMSA derivatives, as well as intercalating antitumor drugs, is the suggestion that DNA binding affinity is related to the

capacity of the involved chromophore to act as a CT acceptor from nucleic acid bases.[33] Photoactivation studies have demonstrated CT between aminoacridines and DNA.[34] mAMSA and other members of this class bind tightly to double-stranded DNA via intercalation,[35,36] a property thought to be important for biological activity.[35] Also, these agents cause DNA strand breaks and chromosomal damage,[35,36] features commonly associated with oxy radicals.[37] The length of time at the active site appears to be important.[33,35] This has been rationalized in terms of long-lived blockage to the passage of replication and/or transcription enzymes. According to the present approach, prolonged binding is required for the generation of oxidative stress. The 9-aminoacridine-4-carboxamide (**4**) possesses antitumor properties similar to those of mAMSA.[33]

The anticancer properties of isoquinolinium salts have been correlated with the presence of the iminium site, in keeping with the theoretical base.[38] Other agents possessing anticancer properties apparently act through redox cycling modes.[19a,30,39–41] Rhodamine 123 also incorporates the iminium cation (see Quinones and Derivatives). In a discussion of CT mechanisms for antimalarial action, the favorable energetics associated with protonation of quinacrine, chloroquine, and quinine on the acceptor properties of these molecules were pointed out.[42] Evidence supports free radical mechanisms for 4-aminoquinolines, e.g. chloroquine, and 9-aminoacridines, e.g. quinacrine.[14]

An additional route for oxy radical formation from mAMSA entails participation of oxidative metabolites[24,43] (see Quinones and Derivatives). Alternatively, interference with normal electron transfer or with the action of DNA topoisomerase II[36,44] may be involved. Virtually all of the current clinical and experimental antitumor agents have been proposed to act via disruption of nucleic acid metabolism at some level,[45] and a common mechanistic pathway is thought to exist for the diverse types



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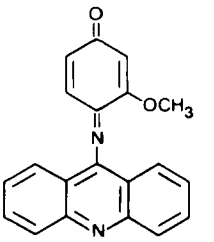

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of agents.[46] Interactions of the drug with thiols, e.g. thiol proteins, essential for normal cell functioning, may also contribute to the cytotoxic effects.[44] The mechanism by which these agents produce their antineoplastic effects is not completely understood.[24,36,44]

The iminium concept appears applicable to a wide variety of biologically active agents.[7,8,11–17]

Quinones and Derivatives

Quinones and derivatives are found in natural systems and are important as xenobiotics.[47] Their function in chemical reactions generally entails redox cycling.[18,47] In more recent times quinone antibiotics have been used in cancer treatment.[19a,48–50]

TABLE II
Cyclic voltammetry of rhodamine 123 (**5**) and nickel(II) salicylaldoximate (**6**)[a]

Compound	Solvent	$-E_{1/2}$ (V) Pt	$-E_{1/2}$ (V) HMDE
Rhodamine 123 (**5**)	DMF	0.54[b]	0.53[b]
Nickel(II) salicylaldoximate (**6**)	DMF	0.91[c,d]	0.91[c,d]
	DMSO	0.92[c,d]	0.90[c,d]

[a] Substrate (0.5 mM), TEAP (0.1 M),vs. NHE.
[b] Reversible at all scan rates (20-200 mV/s).
[c] Reversible at higher scan rates employed ($\geqslant$ 100 mV/s).
[d] 100 mV/S.

1. Diiminoquinones An additional mechanistic possibility involving CT exists for mAMSA derivatives. mAMSA is metabolized *in vitro* by a hepatic microsomal enzyme system producing two oxidation products, mAQDI (**8**) (diimine) and mAQI (**9**) (iminoquinone).[43] Both metabolites are more cytotoxic to L1210 leukemia cells *in vitro* than mAMSA, indicating that bioactivation to form the active species may be important.[43] Interestingly, electrochemical oxidation of mAMSA involves two electrons in a reversible process forming **8** at a formal potential of -0.099 V.[24] Data indicate participation of copper(II) and oxygen in the overall process.[24,51] The intercalated diimine moiety (**8**), complexed with copper (I), apparently generates oxygen free radicals in a redox reaction, resulting in DNA scission.[24] A related system involves the carcinogen benzidine which is converted to benzidinediimine.[52-54]

2. Iminoquinone methides Recently, attention has been paid to rhodamine derivatives, carbon analogues of iminoquinones, as potential anticancer agents. The cationic fluorescent dye rhodamine 123 (**5**) is an example. Results of electroreduction in DMF are presented in Table II. CV of **5** exhibited two reduction peaks, the first of which is reversible with $E_{1/2}$ values of -0.54 V at Pt and -0.53 V at HMDE. Reversibility is supported by constant cathodic peak and half-wave potentials.[25b] The ΔE_p ($\sim$ 60 mV) and $|E_p - E_{p/2}|$ ($\sim$ 60 mV) values were close to theoretical for a one-electron reversible process.[25a,b] Calculated i_{pa}/i_{pc} values were close to unity, indicating stable product formation.[25a] The second irreversible wave occurred at about -1.31 V (100 mV/s). The related rhodamine B was found to reduce irreversibly in aqueous media with $E_{1/2}$ ranging from -0.56 to -0.86 V (pH 6.8 to 12.0),[55] similar to our value for rhodamine 123.

The reversibility and the favorable potential of rhodamine 123 support the feasibility of participation in redox processes, such as interference with normal electron transfer or oxy radical formation. The iminium ion itself is an electroactive site. The positive charge is necessary for anticancer activity.[56,57] Uncharged analogues do not interrupt energy metabolism and are without effect on mitochondria and the cell cycle.[56] This property has been linked to the necessity of the iminium salt for translocation of the dye in respiring mitochondria.[57] Cytotoxicity has been attributed to disruption of normal mitochondrial function, such as translocation of adenosine diphosphate, proton ejection, or electron transport.[56,58] Oxy radical species are integral to the electron transport chains of mitochondria.[19b] According to our working premise, CT properties of **5** could play a crucial role. Structurally related triarylmethane dyes, e.g., parafuchsin, which intercalate,[59] presumably damage DNA via formation of activated oxygen.[60]

In relation to other physiological effects of rhodamine 123, there is selective toxicity

to carcinoma cells *in vitro*[58,61] and *in vivo*,[62] and specific binding to mitochondria in living cells.[56] In fact, carcinoma cells, when treated with **5**, specifically concentrate and retain the drug in their mitochondria, whereas accumulation is less in nontumorigenic cells whose mitchondria lose the dye very rapidly.[57,61] The dye inhibits protein synthesis, but has no affect on DNA or RNA synthesis.[63]

3. Iminoquinones Iminoquinones have not been studied as extensively as quinones.[8] Representatives of this group are 5-iminodaunorubicin,[39] actinomycin D[19a,64] and anthrapyrazoles.[65,66] Pertinent properties include intercalation, oxy radical generation, DNA cleavage, and oxygen dependency. However, redox cycling and radical generation are more difficult with these types.[39]

Metals

Metal species produce a number of responses in biological systems relevant to the present discussion, namely oxy radical generation,[67] DNA strand cleavage,[68] and DNA complex formation.[68,69] Moreover, electron transfer has been suggested to play an important mechanistic role.[70]

As part of our study, electroreduction of nickel(II) salicylaldoximate (**6**) was explored (Table II). CV of **6** in DMF, at Pt or HMDE, produced several peaks, the first of which is reversible at higher scan rates, with $E_{1/2} = -0.91$ V in both cases. Constant cathodic peak and half-wave potentials were observed, and $|E_p - E_{p/2}|$ and ΔE_p values were close to theoretical for a one-electron reversible process.[25a,b] No clear backward sweep wave occurs at a scan rate of 20 mV/s, and i_{pa}/i_{pc} values decrease with decreasing scan rate. Evidently, a slow irreversible reaction occurs after electron uptake. The second peak occurs at about -1.46 V. Analogous results were obtained in DMSO.

The *in vitro* antitumor activities of salicylaldoxime chelates with metals of the first transition series have been described.[21] Of these, the copper complexes demonstrate the highest cancer inhibiting power. Under our conditions, copper(II) salicylaldoximate was found to reduce at -0.51 V (HMDE) and -0.62 V (Pt).[8] The nickel complex (**6**) possesses weaker antitumor potency[21] and takes up electrons at more negative potentials (Table II), thus pointing to a plausible relationship between E_p and activity.

Other metal coordination compounds display anticancer properties. Ammine ruthenium complexes have shown good antitumor potency and are thought to exert their action via redox cycling and accompanying formation of toxic oxygen species which induce DNA strand cleavage.[71] *cis*-Diamminedichloroplatinum(II) (*cis*-DDP), the most prominent anticancer agent in this category has been proposed to act via CT.[8] Other discussions of metal complexes relevant to the present study are available.[8] Furthermore, metal species are known to induce various illnesses.[72].

There are many classes of radiosensitizers, e.g., paraquat, nitroheterocycles, 9-anilinoacridines, barbiturates, quinones, *cis*-DDP, and Cu and Co compounds.[73,74] Since CT (electron affinity) seems to be a reasonable pathway for these types,[74] additional support is provided for electron transfer by the related agents in the present study.

Other considerations

Prior reports point to a relationship between electrochemical properties and physiological activity in other systems.[11–13,75–79] *In vitro* conditions are known to influence

electrochemical processes.[80,81] Several studies indicate that reduction processes *in vivo* may well be better than *in vitro*.[82–84] Also immobilization by binding at the active site should favor electron transfer since *in vitro* reactions, such as dimerization, would be less likely.

Absolute correlation between *in vitro* electrochemical behavior and physiological activity is not reasonable due to the many variables involved *in vivo*, e.g., metabolism, stereochemistry, diffusion, solubility, cell permeability, and active site binding. Given the various possible modes of action for anticancer agents, the CT-oxy radical pathway could conceivably operate in concert with other mechanisms, such as enzyme inactivation and inhibition of DNA replication or synthesis. Also, for some drugs, e.g., 5-fluorouracil,[85] there is no obvious fit to our theoretical framework.

A companion study has been made of related compounds belonging to these same anticancer classes.[86]

Acknowledgments

We thank Dr. Ven Narayanan and Dr. William A. Denny for their generosity in supplying the acridine derivatives.

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Accepted by Dr. B. Halliwell