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The possible role of electron-transfer complexes in the antitumour action of amsacrine analogues

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Amsacrine is a DNA intercalating agent which is active against a number of tumours in mice and is used for the treatment of leukaemia in humans. In its DNA-bound form, amsacrine efficiently quenches the fluorescence of ethidium. Fluorescence lifetime studies demonstrate two populations of DNA-bound ethidium. The first, whose fluorescence lifetime is constant at approx. 3 ns and whose proportion increases with increasing amsacrine binding ratio, may comprise molecules bound in close proximity to amsacrine. The second, whose fluorescence lifetime is longer and variable (10–24 ns) and whose proportion decreases with increasing amsacrine binding ratio, may comprise molecules three or more base-pairs away from ethidium. Studies with a number of derivatives of 9-anilinoacridine containing different anilino substituents suggest that the observed wide variation in quenching capacity is correlated with the magnitude of the substituent dipole moment in a particular direction. Consideration of the geometry of the DNA-binding complex indicates that the negative pole of a dipole established in the anilino ring is directed towards a positively charged site on the ethidium molecule. Quenching of ethidium fluorescence may therefore occur where an electron-transfer complex has formed between ethidium and amsacrine molecules. To ascertain whether electron-transfer complex formation is biologically important in the amsacrine series, ethidium quenching has been quantitated and compared with activity against a transplantable neoplasm in mice, the Lewis lung carcinoma. Compounds which strongly quench ethidium fluorescence are in general highly active antitumour agents. The results are discussed in terms of a model where amsacrine has both a DNA-binding and a protein-binding domain, the latter possibly interacting by formation of an electron-transfer complex. The most likely protein-binding domain is on the enzyme topoisomerase II, the target for its cytotoxic activity.

1. Introduction

Charge-transfer complexes have long been postulated as important in biology, the role of flavin and nicotinamide derivatives in electron-transfer processes in mitochondria being well known [1]. Electron-transfer complexes, because they act over distances larger than those of covalent or hydrogen bonds, may play an important role in the interactions between macromolecules. Although the majority of investigations on electron transfer have been carried out on membranes [2], an increasing number of studies now concern reactions on DNA. DNA stimulates electron-transfer reactions between the excited state of ethidium and some transition metal ions [3] and between ethidium and methyl viologen [4]. Purugganan et al. [5] have also demonstrated that DNA accelerates electron transfer between metal complexes bound to DNA. Since DNA is an important target for the action of antitumour agents, these studies could have particular relevance to the design of new anticancer drugs. The purpose of this communication is to review the evidence for electron-transfer complex formation in a series of compounds related to the clinical antileukaemia agent

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amsacrine, and to consider the possible role of such an electron-transfer complex in its antitumour action.

Amsacrine (*m*-AMSA) is a derivative of 9-aminoacridine first synthesized by Cain and Atwell [6] and used clinically in combination with other antitumour agents for the treatment of acute leukaemia [7]. Amsacrine binds to DNA by intercalation [8] and its mode of cytotoxic action involves the poisoning of the DNA-associated enzyme topoisomerase II [9]. This enzyme functions by promoting the passage of one double strand of DNA through another, a function particularly important during the process of DNA replication, transcription and recombination [10]. In the presence of amsacrine, the dimeric enzyme is maintained in a transition state where both strands of the DNA are broken with one strand covalently attached to each enzyme monomer [11]. The mechanism by which amsacrine maintains this state is still unclear. Studies have been carried out on a series of derivatives of amsacrine (see structure in fig. 1) where either the anilino ring or the acridine chromophore is substituted in various ways [12,13]. There is a good correlation between in vitro activity and the binding of the acridine chromophore [13] but this relationship does not hold for anilino-substituted variants, one of the most notable being *o*-AMSA [14], a biologically inactive isomer of amsacrine in which the methoxy group is at the 2′- rather than the 3′-position. Based on these observations, the hypothesis has been proposed that the acridine portion of amsacrine binds to DNA, while the anilino portion projects from the DNA and binds to another macromolecule [15]. The binding properties of the anilino moiety of amsacrine are therefore of particular interest with regard to its action. Experiments described in the following sections emphasise the interesting properties of the anilino moiety and how they may affect biological activity.

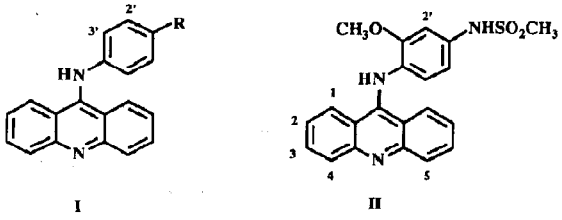

Fig. 1. Structures and numbering system of the free bases of 9-anilinoacridine (I; R = H) and amsacrine (II).

2. The interaction of amsacrine and ethidium on DNA

Both amsacrine and ethidium are DNA intercalators with unwinding angles of 20.5 and 26°, respectively [8]. The planes of the phenyl groups of ethidium [16] and amsacrine [17] in isolated crystals are almost orthogonal (about 70°) to the planes of the corresponding chromophores, suggesting that in the DNA intercalation complex they have the correct geometry to project into one of the grooves of the DNA double helix. Whether this groove is the major or minor groove is unknown, but on the basis of the geometry of binding of ethidium in a dinucleotide complex [16], or the binding of 9-aminoacridine in oligodeoxynucleotide complexes [18] and from theoretical calculations [19], the phenyl group would be positioned in the minor groove. Because of nearest-neighbour exclusion [8], amsacrine in its closest approach to ethidium wil be separated from it by two base-pairs. The amsacrine anilino group has an area of high electron density [14] while the ethidium phenyl group has areas of low electron density [20]. The possibility thus exists for interaction between amsacrine and ethidium, as shown in diagrammatic form in fig. 2.

3. Quenching of ethidium fluorescence by amsacrine

When amsacrine is added to solutions containing ethidium-DNA complexes, the fluorescence of ethidium decreases to a greater extent than expected from displacement alone (fig. 3) [21,22]. The magnitude of the effect varies with the base composition of the DNA, being greatest with poly[dA-dT] [21], and suggesting that some form of fluorescence quenching of the bound ethidium molecules in the presence of DNA-bound amsacrine is occurring. Substituents at other positions

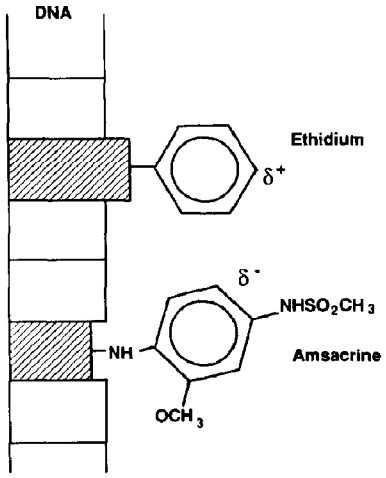

Fig. 2. Diagrammatic representation of ethidium and amsacrine when they are intercalated in DNA at nearest approach (i.e., separated by two base-pairs). For clarity, the groove of the double helix has been 'straightened' to indicate the relative positions of the side chains.

on the amsacrine molecule modify the degree of quenching of ethidium fluorescence, with electron-donating groups on the anilino moiety tending to increase quenching and electron-donating substituents on the acridine chromophore tending to decrease it [22,13].

Fluorescence quenching by amsacrine has also been analysed following laser pulse excitation [23].

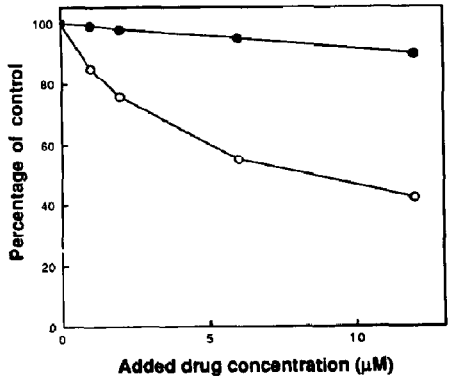

Fig. 3. Relationship between amount of ethidium bound to poly[dA-dT] (●———●) and ethidium fluorescence (○———○) at different concentrations of added amsacrine. Redrawn from published data [21] using 20 μM poly[dA-dT] and 0.01 ionic strength buffer, pH 7.0.

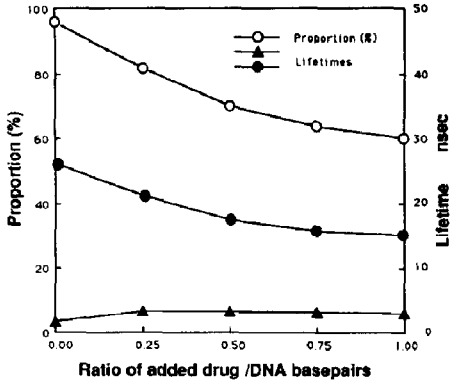

Fig. 4. Fluorescence lifetimes of long (●———●) and short (▲———▲) ethidium fluorescence lifetimes at different concentrations of added amsacrine up to 20 μM in a solution of poly[dA-dT] (20 μM in base-pairs) in 0.01 ionic strength buffer, pH 7.0. (○———○) Proportion of the long lifetime component. Redrawn from published data [23].

Two-component fluorescence lifetimes, one short (3 ns) and one long (14–25 ns) can be resolved in ethidium-poly[dA-dT] mixtures (fig. 4). The lifetime of the short component is, within experimental error, independent of the amsacrine/DNA binding ratio, although its proportion increases with increasing binding ratios. The lifetime of the long component decreases with increasing amsacrine/DNA binding ratios (fig. 4). Fluorescence lifetime measurements for two other analogues of amsacrine, one lacking the methoxy group and the other lacking both methoxy and methanesulphonamide groups, indicate the same pattern of short and long fluorescence components as observed with amsacrine, but with higher drug/DNA binding ratios required for the same result [23].

These results indicate that there are two distinguishable populations of DNA-bound ethidium in the presence of amsacrine. The most likely explanation for the origin of these populations is that the short lifetime component comprises ethidium molecules bound two base-pairs away from amsacrine as in fig. 2, while the long lifetime component comprises molecules situated three or more base-pairs from amsacrine, the fluorescence of which is quenched by a less efficient process.

4. Mechanism of fluorescence quenching by amsacrine analogues

One possible mechanism for ethidium quenching involves resonance energy transfer in which the energy emitted by the excited ethidium is absorbed directly by a second chromophore [24,25]. This cannot occur with amsacrine, since its absorption spectrum does not overlap with the emission spectrum of ethidium [22]. A second possible mechanism which has been proposed to explain the quenching of ethidium fluorescence in water [26] involves the reversible donation of an H^+ by one of the amino groups of the excited ethidium species to an appropriate acceptor molecule (water). The resulting electronically neutral molecule would decay to the ground state by a radiationless process (i.e., without emission of significant visible light) then re-accept a proton from the solvent to return to the original form. This mechanism is unlikely to operate for DNA-bound drugs, since DNA intercalation effectively shields the ethidium amino groups from water molecules [25].

A third mechanism, proposed for ethidium fluorescence quenching on DNA, involves the reversible transfer of electrons between ethidium and amsacrine [21,23]. The excited ethidium molecule accepts an electron from amsacrine and the resulting electronically neutral species decays to the ground state by a radiationless process. It then returns the electron to its donor to recover its original state. Electron transfer to and from ethidium, which has been described in several processes [3,4], is greatly facilitated by excitation of the molecule by visible light [2].

A slight modification of the above mechanism involves the formation of an electron-transfer complex between DNA-bound amsacrine and ethidium molecules when they are in the relationship indicated in fig. 2. The fluorescence of ethidium molecules in such complexes could be quenched because of the electron shared with amsacrine. Such a mechanism would account for the presence of a discrete population of ethidium molecules whose fluorescence lifetime is constant and whose proportion varies with the amsacrine/ethidium binding ratio [23].

The cause of the changes in fluorescence lifetime of the long lifetime component (fig. 4) is not explained by the above hypothesis. A DNA molecule containing ethidium molecules, the fluorescence of some of which is highly quenched, is formally similar to a DNA molecule containing a mixed population of ethidium and nitroethidium (non-fluorescent) molecules [25]. In the latter case, fluorescence is decreased as the ratio of nitroethidium increases, but the measured changes in fluorescence lifetime are not well explained by resonance energy transfer [25]. An alternative explanation is that electron transfer between ethidium molecules and conducted along the DNA molecule [2,5] is responsible for fluorescence quenching.

5. Quenching of ethidium by 9-anilinoacridines: relationship to dipole moments

In the intercalation models for amsacrine, the anilino group does not project perpendicularly from the chromophore and can thus occupy two alternative positions in the groove, one slightly above and one slightly below the plane through the centre of the chromophore (cf. fig. 2). Efficient electron-transfer complex formation with ethidium requires an interaction between the two side chains. The *para* position of the ethidium phenyl has a low electron density as deduced from atomic charge calculations [20]. An interaction between this phenyl group and an electron-rich position on the anilino group of amsacrine could therefore orient the two groups in the close-approach conformation. Since electron density calculations are not available for amsacrine analogues, an indication of such an interaction must be obtained from the electronic properties of anilino substituents on the 9-anilinoacridine molecule.

Derivatives of 9-anilinoacridine in which the anilino moiety is substituted with different groups vary considerably in their ability to quench the fluorescence of DNA-bound ethidium [22] (table 1). Compounds with electron-donating substituents (e.g., amino, hydroxy) on the anilino ring have high fluorescence quenching efficiencies [22]. Since the acridinyl group would be expected to be

charged in many of these complexes when it is bound to DNA [22] and therefore electron withdrawing, a dipole would be set up across the anilino ring. However, a compound such as that with an $NHCOCH_3$ substituent does not donate electrons but still quenches ethidium fluorescence (table 1). The $NHCOCH_3$ substituent, although not electron donating, has a high group dipole moment (table 1), indicating a high electron density almost at right angles to the substituent bond axis. The $NHSO_2CH_3$ group, which by itself confers good quenching efficiency [23], has a strong dipole (about 3 Debye), but its direction is uncertain and outside the plane of the anilino ring [22,27].

Table 1

Physicochemical and biological properties of 9-anilinoacridine derivatives

Substituent [a]	Dipole moment [b]		$Q_{0.1}$ [c] (%)	OD [d] (mg/kg)	ILS [e] (%)
	μ	θ			
NH_2	1.53	49	43	65	117
$NHCH_3$	1.71	40	51	45	48
$N(CH_3)_2$	1.58	30	44	65	100
OH	1.55	90	19	150	23
OCH_3	1.28	72	19	65	7
CH_3	0.37	0	3	65	12
H	0	0	3	65	12
$NHCOCH_3$	3.69	100	11	65	31
F	1.47	180	3.5	–	ND [f]
Cl	1.59	180	3.5	65	0
Br	1.57	180	9	–	ND
I	1.4	180	9	–	ND
$CONH_2$	3.65	110	6	–	ND
$COOCH_3$	1.83	110	6.5	–	ND
$COCH_3$	2.96	132	5	–	ND
CN	4.05	180	5.5	–	ND
NO_2	4.01	180	7	–	ND

[a] Substituent (R) on anilino ring of 9-anilinoacridine (see fig. 1).

[b] Magnitude (μ) and angle (θ) of group dipole moment [27].

[c] Quenching of ethidium-poly[dA-dT] fluorescence with ethidium and drug binding ratios adjusted to 0.1 drug molecules/base-pair [13].

[d] Optimal intraperitoneal dose injected on days 5, 9 and 13 after intravenous inoculation of 10^6 Lewis lung cells [29].

[e] Percentage increase in lifespan as compared to untreated tumour-bearing mice. Values of less than 40% are considered to be non-significant [29].

[f] Not determined, but expected to be inactive on the basis of in vitro and in vivo testing with L1210 leukaemia (ref. 12 and unpublished data).

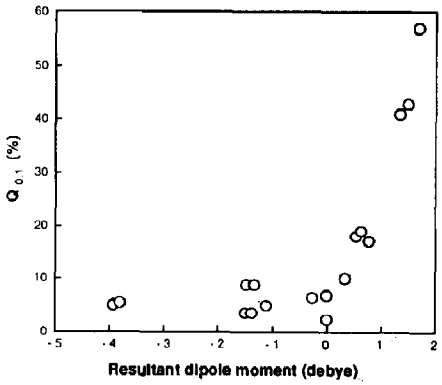

Fig. 5. Relationship between resultant group dipole moment, calculated at an angle of 20° to the substituent axis of the anilino ring of 9-anilinoacridine, and the degree of ethidium fluorescence quenching ($Q_{0.1}$) for the compounds listed in table 1. $Q_{0.1}$ values were measured with poly[dA-dT] (20 μM) in 0.01 ionic strength buffer, pH 7.0, using the method of Baguley and Cain [13].

If a dipole, orientated with the negative end away from the acridine, is important in determining the magnitude of DNA-bound ethidium quenching [22], there may be an observable relationship between group dipole moments and ethidium fluorescence quenching. Dipoles have direction as well as magnitude, making it possible to determine the optimal direction required for fluorescence quenching using the range of 9-anilinoacridine derivatives. Calculation of the resultant dipole moment for the compounds in table 1 using an (arbitrary) angle of 20° to the *para* axis of the anilino ring provides a good relationship with fluorescence quenching (fig. 5). The anilino group may therefore serve two functions, the first to orientate the side chain in the DNA close to ethidium, and the second to donate electrons to form an electron-transfer complex with ethidium.

6. Quenching of ethidium fluorescence by other DNA-binding molecules

Quenching of ethidium fluorescence has been described for many compounds including transition metal ions [3], methyl viologens [2], non-fluo-

rescent ethidium derivatives [25] as well as with derivatives of ellipticine [21], phenazine and anthracenedione (B.C. Baguley, unpublished data). Several explanations for quenching involving electron transfer have been advanced [2,3,21].

The antitumour compound ellipticine does not itself quench ethidium fluorescence, but 9-hydroxyellipticine [21] and 9-aminoellipticine (result not shown) quench at about 50% of the efficiency of amsacrine. The absence of a spectral overlap between the emission spectrum of ethidium and the absorption spectrum of the ellipticine precludes resonance energy transfer quenching. The combination of a charged nitroheterocycle at one end of the ellipticine ring system and an electron-donating substituent (hydroxy or amino) at the other end would provide a strong dipole which might interact with ethidium by dipole-dipole interaction, facilitating the formation of an electron-transfer complex. In a series of antitumour phenazine derivatives containing a positively charged side chain [28], low-efficiency quenching of ethidium fluorescence was noted for compounds having one or more methoxy groups on one side of the phenazine chromophore (result not shown). Again, a strong dipole would be formed which could interact with ethidium. The anthracenedione derivative mitoxantrone is an antitumour DNA intercalator which also quenches ethidium fluorescence (B.C. Baguley, unpublished data). It may be expected to have a dipole between the positively charged side chains and the chromophore. However, since its absorption spectrum overlaps with the fluorescence emission spectrum of DNA-bound ethidium, resonance energy transfer could account for quenching and no conclusion can be made as to whether electron-transfer complexes are forming.

7. Significance of electron-transfer complexes for biological activity

Ethidium quenching may constitute an index of the ability of the 9-anilinoacridine molecule to form electron-transfer complexes with biological macromolecules as well as with ethidium. If this is so, the antitumour activity of these drugs, which can be measured by inoculating mice with tumour cells and at various times later treating with drug [29], might be related to their ability to form complexes which quench DNA-ethidium fluorescence. An early study on the antitumour activity of a series of amsacrine analogues containing electron-donating or electron-withdrawing groups on the anilino ring [14] concluded that a high electron density at the 6′-position (*ortho* to the methanesulphonamide group) is required for antitumour activity, consistent with this hypothesis. More recently, antitumour data for a large number of amsacrine analogues have been collected in this laboratory [29–33] and quenching of ethidium has quantitated by determining $Q_{0.1}$ values, defined as the percentage quenching of ethidium fluorescence under conditions where both drug and ethidium have a binding ratio of 0.1 molecules/base-pair [13]. Compounds with both high $Q_{0.1}$ values and high biological activity include CI-921, a disubstituted amsacrine derivative now undergoing clini-

Table 2

Physicochemical and biological properties of amsacrine and analogues

Compound [a]	K [b] (M^{-1}) ($\times 10^{-6}$)	$Q_{0.1}$ [c] (%)	OD [d] (mg/kg)	ILS [e] (%)
Amsacrine	0.37	31	13.3	42
CI-921	1.15	45	20	167
1′-$NHSO_2CH_3$	1.08	16	150	60
1′-$NHSO_2$-phenyl	1.14	17	3.9	4
1′-$NHSO_2CH_3$, 3′-$NHCH_3$	2.63	42	30	80
1′-$NHSO_2CH_3$, 3′-$N(CH_3)_2$	0.22	34	100	192
1′-$NHCOOCH_3$	1.66	19	30	60
1′-$NHCOOCH_3$, 3′-$NHCH_3$	2.24	48	45	112

[a] Amsacrine (1′-$NHSO_2CH_3$, 3′-OCH_3) has the structure shown in fig. 1. CI-921 is the 4-CH_3, 5-$CONHCH_3$ disubstituted derivative of amsacrine. Other compounds are variants of the anilino substitution pattern of amsacrine.

[b] Association constant for poly[dA-dT], determined at 0.01 ionic strength and pH 7.0, as previously described [22].

[c] Quenching of ethidium-poly[dA-dT] fluorescence with ethidium and drug binding ratios adjusted to 0.1 drug molecules/base-pair [13].

[d] Optimal intraperitoneal dose injected on days 5, 9 and 13 after intravenous inoculation of 10^6 Lewis lung cells [29].

[e] Percentage increase in lifespan as compared to untreated tumour-bearing mice. Values of less than 40% are considered to be non-significant [29].

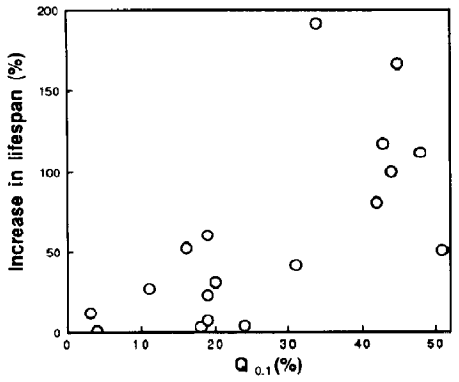

Fig. 6. Relationship between the percentage increase in lifespan of mice with Lewis lung tumours induced by the optimal dose of anilinoacridine derivatives and their ability to quench DNA-bound ethidium fluorescence. Life extension data from tables 1 and 2 are either previously published [30–33] or determined using published methods [29].

cal trial [30,34], as well as compounds with more powerful electron-donating groups on the anilino ring [31–33]. Data for some of these compounds are listed in table 2. Data for tables 1 and 2 are plotted in fig. 6 and show a reasonable degree of linear correlation ($r = 0.61$; $p < 0.01$). Since there are a large number of factors which influence antitumour activity of amsacrine analogues, including their ability to distribute in tissues [35] and their susceptibility to the phenomenon of multidrug resistance [36], the scatter of data points in fig. 5 is quite acceptable.

It is interesting to note that for a series of amsacrine derivatives [13], as well as for other series of analogues studied in this laboratory, dose potency (the dose of drug required for optimal activity) is positively correlated with a number of drug physicochemical properties, the most important of which is the magnitude of the DNA association constant. In contrast, antitumour activity (which measures the selective cytotoxicity for tumour vs. host tissues) is not correlated with the DNA association constant. The result in fig. 6 suggests that the electronic properties responsible for ethidium fluorescence quenching are important for selective cytotoxicity, which might in turn be related to the target for cytotoxicity in the tumour cell.

8. Concept of DNA- and protein-binding domains in the action of amsacrine derivatives

As discussed in section 1, topoisomerase II is thought to be the target of action of compounds in the amsacrine series. Topoisomerase II is a dimeric enzyme which can exist in a DNA-bound form in either of two states, the 'non-cleavable complex' and the 'cleavable complex' [10]. The latter form, where a double-stranded DNA break is masked by DNA-protein cross-links through a phosphotyrosine linkage, is the form which is stabilised by the presence of amsacrine and its analogues [10,37]. It is thought that the persistence of this form of the enzyme leads to irreversible DNA breakage and cell death [38]. If amsacrine binds selectively to and stabilises the cleavable complex form of the enzyme, this would give rise to its observed cytotoxic effects.

Amsacrine has been postulated on the basis of structure-activity relationships to have both a DNA-binding domain (the acridine chromophore) and a second macromolecular binding domain (the anilino group) [12,15]. If this second binding domain is situated on the topoisomerase II enzyme, one might expect that changes in the structure of this enzyme would be reflected in changes in the structure of the anilino moiety of amsacrine required for optimal activity. Murine P388 cells with an altered DNA topoisomerase II enzyme are highly resistant to amsacrine, as well as to a number of other topoisomerase II-directed drugs [39]. The degree of cross-resistance of this line to amsacrine derivatives varies only slightly for analogues containing different acridine substitution patterns, but varies from more than 70-fold to less than 2-fold for analogues containing anilino groups with different substitution patterns [39a]. This observation suggests strongly that amsacrine binds to topoisomerase II as well as to DNA. Etoposide, another drug with the same enzyme target, is thought to have a binding site on the topoisomerase II molecule [40], and has recently

been hypothesised to have a DNA-binding domain as well [41]. The concept of DNA- and protein-binding domains is well established for gene activator proteins, which have a DNA-binding polypeptide segment which binds selectively to a DNA site known as the enhancer sequence, and a second polypeptide domain (generally anionic) which binds to a transcription factor in the eukaryotic RNA polymerase complex [42].

Circumstantial evidence from both theoretical considerations and ethidium-binding studies suggests that the ability of amsacrine derivatives to form a charge-transfer complex is important for their antitumour activity. The binding energies of such complexes, typically a few kcal/mol [43], emphasise their potential importance in protein binding. The electron acceptor for such a complex could be a histidine residue [43] or a protein-associated metal ion or chromophore. Hydrogen bonding and van der Waals contacts would presumably also be important in this interaction, and the role of steric factors has already been established, since the isomeric compound *o*-AMSA is considerably less active as a topoisomerase inhibitor [9] and other analogues containing 2'-substituents are generally inactive as antitumour agents [14].

Amsacrine is readily oxidised electrochemically [44,45] and oxidation potentials of amsacrine analogues correlate positively with antitumour activity [44]. There have been a number of suggestions that oxidation of topoisomerase II inhibitors leads to free radical-mediated cytotoxicity [44–48]. The possibility that such drugs, after oxidation, react covalently with the topoisomerase II enzyme has also been considered [48]. The ability of amsacrine to form reversible electron-transfer complexes is difficult to distinguish from that to participate in irreversible chemical reactions, since similar electronic properties are needed for both. However, the observation that amsacrine is equally active in oxic and hypoxic environments [49], together with the demonstration that no detectable levels of oxidation products of amsacrine are generated in cultured cells [50], argues against the oxidation of amsacrine as a necessary prerequisite for activity. Further work is required to resolve whether non-covalent interaction (ternary complex formation) or covalent interaction mediates the cytotoxic action of amsacrine.

9. Conclusions

The availability of laser pulse techniques has allowed the electronic interactions between the antitumour drug amsacrine and another DNA intercalating agent, ethidium, to be studied. This interaction varies independently of DNA association constant but may be dependent on DNA-binding geometry, since it varies with DNA of different base composition [21]. The degree of interaction appears to be related primarily to the electron-donating and dipole-forming capacity of the anilino moiety of the amsacrine molecule. Many compounds with high fluorescence quenching properties have high activity against the Lewis lung tumour, a mouse tumour that is resistant to many clinical agents [29]. Although the evidence that electron-transfer complexes are involved in the antitumour action of amsacrine is inferential, the results point to the importance of further work to establish whether such complexes have a role in drug-receptor interactions, and in particular whether they are involved commonly in drugs which target the enzyme topoisomerase II.

All of the main classes of clinical antitumour drugs thought to have this enzyme as a target (the anthracyclines, amsacrines, anthracenediones, ellipticines and epipodophyllotoxins) have easily oxidisable or reducible groups, and the reason for this is unknown. It has been hypothesised that such drugs may react chemically at their site of action, and whereas this may be true in some cases, the alternative hypothesis, that they promote the formation of electron-transfer complexes to stabilise the cleavable complex form of topoisomerase II, should also be considered.

The results provide a new principle for the design of DNA-binding antitumour agents. Such compounds should have two macromolecular binding domains, one for DNA and the other for a protein such as topoisomerase II. An increase in binding energy of a few kcal/mol (selective for the cleavable complex form of topoisomerase II)

would not only substantially increase the association constant of amsacrine for its target binding site but also result in the selective stabilisation of the topoisomerase II cleavable complex on the DNA, consistent with the biological data [11]. This principle could be also applied to the design of compounds targetted towards other DNA-binding proteins such as topoisomerase I and gene regulatory proteins. In the same way that gene activator proteins enhance the stability of DNA transcription elements, DNA-binding agents could considerably modify the properties of important DNA-binding proteins.

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