MARIOLA K. PIESTRZENIEWICZ, MAŁGORZATA CZYŻ, WILLIAM A. DENNY * 
and MAREK GNIAZDOWSKI

INHIBITION OF RNA SYNTHESIS IN VITRO BY 9-AMINOACRIDINE 
CARBOXAMIDE ANTITUMOR AGENTS. EFFECTS ON OVERALL 
RNA SYNTHESIS AND SYNTHESIS OF THE INITIATING 
DINUCLEOTIDE * *

Department of General Chemistry, Institute of Physiology and Biochemistry, School of Medicine in 
Lódz, Inndeya 6; 90-131 Lódz, Poland

* Cancer Research Laboratory, University of Auckland School of Medicine, 
Private Bag, Auckland, New Zeland

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A series of 9-aminoacridine carboxamide derivatives of systematically varied 
structure was assayed in an RNA synthesis in vitro system. Escherichia coli 
DNA-dependent RNA polymerase and DNA derived from phage T7 or calf thymus 
were used to measure the effect of the drugs on overall RNA and the initiating 
dinucleotide (pppApU) syntheses. By means of multiple linear regression analysis it 
was shown that the inhibition of these reactions depends both on the drug equilibrium 
binding constant and kinetic parameters of dissociation of drug-DNA complexes.

The inhibition of overall RNA synthesis in vitro, depends on several factors 
and the analysis of a ligand effect on different steps of the reaction includes the 
mode of ligand interaction, i.e. intercalative or non-intercalative binding; 
equilibrium binding strength, DNA sequence-specificity; and the rates of both 
association and dissociation of ligands [1-9]. The kinetics of drug-DNA 
interactions has recently attracted much attention, since a correlation has been 
observed between the rates of dissociation of drugs from DNA and their 

A series of 9-aminoacridine carboxamide derivatives of systematically 
varied structure has recently been described [10, 12, 13]. These compounds 
form intercalative complexes of roughly similar binding strength with DNA. 
Although considerable differences are observed within the series in both the 
cytotoxicity of the compounds and in the kinetics of dissociation of their 
complexes with DNA, these features are well correlated. We considered these
acridines might be valuable tools, to evaluate the contribution of particular physico-chemical factors to their ability to poison the transcriptional template activity of DNA.

Such detailed analysis of the effect of drug action on the transcription of DNA by RNA polymerase may provide information on the enzyme-template interactions [14-18] of this reaction, as well as indicating the modulations which a protein may impose on ligand-DNA interactions [11]. The aim of these experiments was therefore to show whether the differences in dissociation rates of the acridine-DNA complexes are related to the ability of the drugs to affect the level of total RNA synthesis and/or the initiation step.

MATERIALS AND METHODS

Acridine derivatives (Fig. 1) were described [10]. Phage T7 DNA and Escherichia coli DNA-dependent RNA polymerase (EC 2.7.7.6) were isolated as described previously [7] and [19], respectively. Sources for other materials are indicated under refs. [6, 19 and 20]. The effect of acridine derivatives on RNA synthesis with phage T7 and calf thymus DNA and on pppApU synthesis in the presence of T7 DNA were assayed as described before [20], except that the incubation time was 10 min. RNA or the initiating dinucleotide syntheses were assayed at two - four drug concentrations, and expressed as a percentage of the controls containing no inhibitor. Drug concentrations resulting in a 50% decrease in RNA synthesis (IC$_{50}$) or in pppApU synthesis (IA$_{50}$) were read from the inhibition curves.

RESULTS AND DISCUSSION

Analysis of the effects of several DNA-interacting ligands on different steps of DNA-dependent RNA synthesis has led to a general conclusion that the mechanism of their action depends on the rate of dissociation of the complexes they form with the template [5, 8, 21, 22]. Hence ethidium bromide, acridines and diacridines which form rapidly dissociating complexes with DNA [5, 8, 23], preferentially inhibit early steps of RNA synthesis (i.e. binding of RNA polymerase to DNA), while actinomycin D, a slowly dissociating ligand, affects elongation of RNA chains [5, 8, 18]. However, other explanation of the differential effects of the ligands could be proposed. Preferential inhibition of early steps of RNA synthesis in vitro by the antibiotic distamycin A, a tightly DNA-binding non-intercalating ligand, might be ascribed to the ligand affinity to d(A-T) rich promoter region [24]. It can be supposed therefore that the small effect of actinomycin D on early steps of RNA synthesis could be due to its high d(G-C) specificity, and that the base-pairs or sequence specificity is a factor in inhibition of RNA synthesis. More thorough analysis of the effect of
Fig. 1. Structure of 9-aminoacridinecarboxamide derivatives

<table>
<thead>
<tr>
<th>Acridine no.</th>
<th>side-chain position</th>
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ligands on the formation of RNA polymerase-DNA "open" promoter complex which has been undertaken recently [15] has shown that actinomycin D affects this reaction, although to a significantly lower extent than distamycin A.

Most of the data hitherto available were obtained from experiments with ligands differing greatly in structure, in base or sequence specificity and in the steric characteristics of their interactions with DNA. An advantage in using the above 9-aminoacridinecarboxamides in studies of RNA synthesis in vitro is that they represent a new class of DNA-intercalating antitumor drugs with well defined structure-activity relationships [10,12]. In general they are highly polar and their association constants of above $10^7$ M$^{-1}$ for binding to poly d(A-T) and poly d(G-C) are broadly similar although some differences in affinities to these copolymers were noted [10] (see also Table 1). Analysis of the stability of complexes of these 9-aminoacridinecarboxamides with calf thymus DNA revealed considerable differences within the class. Two, three or four transients can be distinguished in the dissociation kinetics. The relationship between the structure of the particular compounds and the kinetic data allowed a model of their highest stability complex with DNA to be proposed [10]. Summarizing, the most stable complexes were formed by compounds where the carboxamide chain with two methylene units was at position 4 of the 9-aminoacridine ring, and the ring either bore no other substituents (compound 3) or had a methoxy group at positions 1-, 2-, 5-, 6- or 7- [10]. Four transients in the dissociation kinetics were resolvable for these compounds [10] (see also Table 1). Most interestingly, these drugs showed high cytostatic effects both in vivo and in cell cultures [10, 12].

The effect of acridines on RNA polymerase was assayed with phage T7 DNA and calf thymus DNA. Although the latter is not a natural template for E. coli RNA polymerase its use in these experiments is substantiated, since the original kinetic data were obtained with calf thymus DNA [10]. The IC$_{50}$ values are shown in Table 1.

As found before for several other acridine, diacridine and triacridine derivatives [4, 6, 7] the IC$_{50}$ values show a dependence on the binding constants and are somewhat higher for calf thymus DNA than for phage T7 DNA. However the dissociation rates of the acridine-DNA complexes may play a role. It is not yet possible to determine whether multiple time constants represent parallel dissociations from a number classes of binding sites of different affinity or if the dissociation is a sequential process, where all of the drug molecules have to proceed through a series of different transient states before final release from the DNA [10]. As, however, there is a positive correlation between in vivo biological activity and the fourth transient in the dissociation kinetics [10], we assumed that the longest lived complex may contribute most significantly to inhibition of overall RNA synthesis. With this assumption, our data have been subjected to statistical analyses. One of the acridines studied (compound 14) exhibited a very low inhibitory effect
Table 1
Physicochemical data for acridines and inhibition of RNA and pppApU syntheses
For the number of the compound see Fig. 1. Log $K_{GC}$ and log $K_{AT}$ are the logarithms of the association constants for bindings to poly d(G-C) and poly d(A-T), respectively. Time constants describing the dissociation profile of calf thymus DNA-drug complexes. IC$_{50}$ and IA$_{50}$ are drug concentrations resulting in a 50% decrease in RNA and pppApU synthesis, respectively.

<table>
<thead>
<tr>
<th>Acridine No.</th>
<th>Log $K^b$</th>
<th>Time constants (min)$^c$</th>
<th>IC$_{50}^d$ (µM)</th>
<th>IA$_{50}^d$ (µM)</th>
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$^a$ The values are taken from Wakelin et al. [10].

$^b,c$ The mean values of three-five (b) or two (c) independent experiments ± SD (b) or ± range values (c) are shown. CT-DNA = calf thymus DNA.
(IC$_{50}$ > 160 µM, for T7 DNA). It has a considerably lower binding constant
than the other acridines (Table 1) and was not included in the following
analysis. Carrying out forward stepwise multiple linear regression of log IC$_{50}$
against log $K_{AT}$ and the indicator variable $I$ ($I=1$ if the T$_4$ transient is
observed, i.e. for acridines 3, 7, 8, 10, 11 and 12, otherwise $I=0$) we obtain, for
the calf thymus DNA data the best-fit equation:

$$\log \ IC_{50} = -0.36(\pm 0.13)\log \ K_{AT} - 0.27(\pm 0.05)I + 4.02$$

$n=13 \quad r=0.92 \quad s=0.08$

where $n$ is the number of compounds, $r$ is the multiple correlation coefficient,
$s$ is a standard error of the estimate and the numbers in parentheses are the
95% confidence limits of the coefficients.

The log AT values and the parameter $I$ representing kinetics of interactions
may be used as independent variables as there is a very low correlation
coefficient between them ($r=0.304$) for the dataset studied. If log IC$_{50}$ values
are plotted against log $K_{AT}$ as one variable much lower correlation coefficient
is obtained ($r=-0.60$).

When the T7 DNA data are subjected to statistical analysis in the manner
indicated above, the first variable to enter the regression is log $K_{AT}$ ($r=-0.73$),
followed by T$_4$ transient if observed ($I=1$) to give the best-fit two-variable
equation:

$$\log \ IC_{50} = -0.71(\pm 0.21)\log \ K_{AT} - 0.21(\pm 0.08)I + 6.44$$

$n=13 \quad r=0.85 \quad s=0.14$

It can be concluded from these analyses that the dissociation kinetics of the
acridines from the template is reflected in their inhibition of overall RNA
synthesis, the inhibitory effects being dependent both on equilibrium binding
constant $K_{AT}$ and the presence of the fourth transient. It is worth mentioning
here that the poly d(AT) equilibrium binding data correlate quite well with calf
thymus binding data in cases where both have been measured [25]. When the
log $K_{GC}$ and transcription data have been similarly analysed the correlation
was much less significant with addition of the $I$ variable making no difference
(not shown).

To check whether there is any dependence of the inhibitory effects of the
acridine-DNA complex decay at the earlier steps of RNA synthesis an assay of
abortive initiation was used. RNA polymerase in the presence of ATP and
UTP repetitively synthesizes pppApU on A1 and A3 promoters of T7 DNA
(see refs. [16, 17 and 20]). The amount of the initiating dinucleoside
tetraphosphate was quantitated following its separation from the substrates by
means of paper chromatography (Fig. 2). Estimations of pppApU in the
uninhibited control and at the different acridine concentrations allowed
determination of the IA$_{50}$ values (Table 1).
Fig. 2. Synthesis of pppApU on T7 DNA or DNA-compound complex. The radioactivity in products which were synthesized in the absence, ●, or in the presence, ○: of compound 3 (10.7 μM) is plotted vs. migration in a water/(NH₄)₂SO₄/isopropanol solvent system.

The statistical analysis dividing the dataset into the same subclasses (i.e. using a dummy variable I = 1 for the acridines displaying the fourth long lived transient T₄ and I = 0 for those which do not form this pattern of kinetics) shows that the parameter accepting most of the variance (r = −0.68) is \( \log K_{AT} \) while introduction of the second variable I gives the best-fit equation:

\[
\log IA_{50} = -0.49(±0.14) \log K_{AT} - 0.24(±0.05)I + 4.83 \\
_{n=13} \quad r=0.90 \quad s=0.09
\]

Except for compound 8 (Table 1) these inhibitory effects are close to the IC₅₀ values found with T7 DNA. Other results were obtained for antracycline antibiotics, which affected the overall RNA synthesis to a greater extent than the synthesis of the initiating nucleotide [16, 17]. Similarly, psoralen adducts to DNA strongly inhibited RNA synthesis while pppApU synthesis was less sensitive [20]. Since it was concluded from the latter studies that both intercalating ligands (daunomycin and marcellomycin) [16, 17] and
furocoumarin adducts [20] influenced early steps of RNA synthesis only slightly, it might be inferred from this study that the acridines affect either binding of the enzyme to the template or the early initiation step. Further experimentation is needed to evaluate the two effects. The results obtained do however indicate that this family of compounds is a valuable tool with which to study the role of kinetic properties of ligand-DNA interactions on particular steps of RNA polymerase catalytical function. The effects observed here show a correlation with the cytostatic activity of the acridines assayed, both at the cellular level and in vivo [10].

REFERENCES