TEMPLATE SPECIFIC INHIBITION OF DNA POLYMERASES FROM RNA TUMOR VIRUSES BY DISTAMYCIN A AND ITS STRUCTURAL ANALOGUES

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1. Introduction

Distamycin A (Dist/A) is an antiviral antibiotic which was shown to be active against several DNA viruses [1–3] and to inhibit the synthesis of adaptive enzymes in bacteria [4]. Experimental studies suggest that Dist/A blocks some early steps in the growth cycle of DNA viruses, probably connected with DNA replication [5]. This mechanism correlates well with the ability of Dist/A to bind to double-stranded and single-stranded DNA [6, 7] and to inhibit the template activity of DNA for DNA [8] and RNA [7] polymerases.

The structure of Dist/A is characterized by 3 residues of 1-methyl-4-aminopyrrole-2-carboxylic acid and 2 side chains (fig. 1). Some structural analogues of Dist/A have been recently synthesized [9] by varying the number of pyrrole residues in the molecule. Chandra et al. [10] have shown that the analogues containing 4 (Dist/4) and 5 (Dist/5) pyrrole rings are more active than the parent compound on vaccinia virus and in the DNA-dependent RNA polymerase reaction. It is known that RNA-oncogenic viruses require DNA synthesis for their replication. This prompted us to investigate the effect of Dist/A and its analogues on the murine sarcoma virus (MSV-M) *in vitro*. The antiviral activity of these analogues was found to be parallel to their extent of inhibition of DNA polymerase activity of RNA tumor viruses.

2. Materials and methods

^{3}H-labeled deoxynucleoside triphosphates were obtained from NEN-Chemical GmbH, Germany. Unlabeled deoxynucleoside triphosphates were purchased from Nutritional Biochemicals Corp., Ohio, USA. Poly (dA–dT) and poly (A) were supplied by Miles Laboratories, Elkhart, Indiana, USA. Poly (dI–dC) was provided by Dr. G. Weimann of Boehringer Mannheim GmbH, Tutzing. Oligo $(dT)_8$ was a kind gift of Dr. E. Lodemann, Frankfurt. The oligodeoxy nucleotide $(dT)_8$ was annealed with poly (A) at 40° for 15 min in a solution containing 200 μg/ml of each component and allowing it to cool slowly at room temp.

MSV-M, kindly supplied by Dr. J.B. Moloney (NCI, Bethesda, Md., USA) was passed by intramuscular injection into suckling Swiss mice, and extracted from the tumor tissue. Friend leukemia virions (FLV) were isolated from infected spleens (AKR mice) and purified by sucrose-density gradient centrifugation. The viral extracts used to assay for DNA-polymerase activity were stored at −70° before use.

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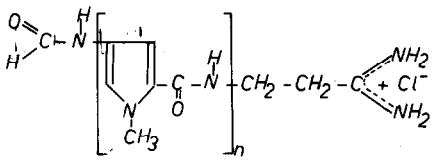

n = 2 Dist/2
n = 3 Dist/A
n = 4 Dist/4
n = 5 Dist/5

Fig. 1. Chemical structures of distamycin derivatives.

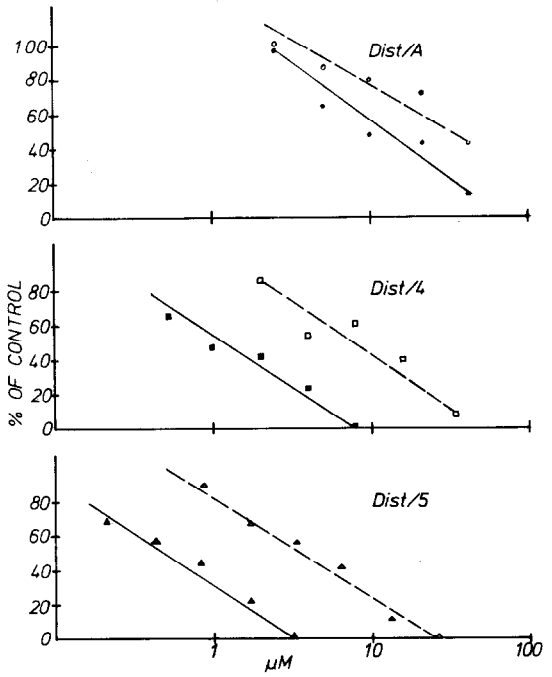

Fig. 2. Activity of distamycin derivatives on mouse embryo cells infected or not with MSV-M. (——): Indicates inhibition of MSV-M foci formation; (— — — —): inhibition of normal cell proliferation. On the abscissa: dose (μM); on the ordinate: % of the corresponding control values.

Antiviral activity assay: the experiments were carried out on mouse embryo fibroblast cultures, according to O'Connor [11]. Cell cultures were infected with MSV at a multiplicity of infection of 0.02 per cell, and treated with various drug concentrations for 3 days after the virus adsorption. Foci were counted 5 days after the infection.

Table 1
Antivital and cytotoxic activities of some distamycin derivatives.

Compound	ID_{50} (μM)		TI
	Cell	MSV	
Dist/A	32.0	12.6	2.5
Dist/4	7.8	1.1	7.0
Dist/5	3.4	0.44	7.7

The activity of distamycin derivatives was tested on mouse embryo cells infected or not with MSV (M) as described under methods. ID_{50} = Inhibiting dose 50%; TI = therapeutic index, ID_{50}-cell/ID_{50}-virus.

DNA-polymerase assay: DNA-polymerase activity was assayed essentially by the method of Ross et al. [12]. The reaction mixture, regardless of template, was similar to that of Ross et al. [12], except that we used 0.04 M Tris and the end concentration of Triton X-100 was 0.02%. The reaction mixture contained 0.2 μg of the template used. The reaction mixtures containing virions (28 μg of protein) were incubated at 37° for 90 min. Acid precipitable material was counted on "Millipore" filters (HAWP 02500) in a liquid scintillation counter. Protein was estimated by the method of Lowry et al. [13].

Cytotoxic activity assay: uninfected cultures, treated parallel to the infected ones, were trypsinized 5 days after the beginning of the experiment, and cells were counted in a hemocytometer.

3. Results and discussion

The results, reported in fig. 2, show that treatment with Dist/A, Dist/4 and Dist/5 reduces the number of MSV-foci produced *in vitro*. The inhibitory activity was dependent on the dose used, and increased according to the number of pyrrole residues in the molecule. The cytotoxic activity of these compounds increases at higher doses; however, it was always less than their antiviral activity.

Since the dose-dependent curves for cytotoxicity and antiviral activity are almost parallel, it was possible to calculate the ratio cell-ID_{50}/virus-ID_{50} (Therapeutic index = TI). As shown in table 1, the TI increased from 2.5 for Dist/A, to 7.0 for Dist/4 and to 7.7 for Dist/5. These data confirm our previous ob-

Table 2
Inhibition of reverse-transcriptase activity of FL-virions by distamycin derivatives in the absence of exogenous template.

System	Antibiotic used*	^{3}H-TMP incorporation into DNA (cpm/reaction mixt.)	% of control
Without virions	–	14	4.1
Without Triton	–	40	11.7
Virions + RNase **	–	61	17.6
Complete	–	343	100
Complete	Dist/2	336	98
Complete	Dist/A	242	70.5
Complete	Dist/4	203	59
Complete	Dist/5	206	59.8

* Antibiotic concentration = 20 μg/reaction mixt.
** Virions containing Triton were preincubated at room temp for 25 min with 50 μg/ml of pancreatic RNase. The reaction conditions are described under Materials and methods.

Table 3
Inhibition of DNA-polymerase activity of FL-virions by distamycin A and its analogues in the presence of various templates.

Antibiotic used*	^{3}H-TMP incorporation into DNA poly (dA–dT) (cpm/reaction mixt.)**	^{3}H-dGMP incorporation into DNA poly (dI–dC) (cpm/reaction mixt.)**	^{3}H-TMP incorporation into DNA poly rA–$(dT)_8$ (cpm/reaction mixt.)**
None	1020 (100)	506 (100)	520 (100)
Dist/2	357 (35.8)	441 (87.5)	450 (85.5)
Dist/A	137 (14)	442 (87.5)	330 (63.5)
Dist/4	117 (11.9)	460 (91)	290 (55.5)

* Antibiotic concentration = 20 μg/reaction mixt.
** The figures in brackets indicate percent of the control. The reaction conditions are described under Materials and methods.

servations [10] on the increase of antiviral activity (vaccinia virus) of Dist/4 and Dist/5, as compared with Dist/A, and show that this class of compounds is endowed with antiviral activity of MSV, an RNA-oncogenic virus whose replication depends on DNA synthesis.

The existence of virion-associated DNA polymerases [14, 15] in oncogenic RNA viruses suggests that information flow from RNA to DNA may be one of the reasons leading to oncogenesis. The search for specific inhibitors of this reaction has obvious implications in the chemotherapy of viral carcinogenesis. Synthetic polymers containing either deoxyribonucleotide or ribonucleoside strands can be used as template by the RNA-dependent DNA polymerase. The activity of distamycin derivatives on the DNA polymerase activities of FLV and MSV-M using various templates was tested.

As shown in table 2 the reverse transcriptase activity (without exogenous template) of FL-virions is inhibited by distamycin derivatives containing 3, 4 and 5 pyrrole rings. The compound containing 2 pyrrole rings (Dist/2) is ineffective, which is similar to our observations on vaccinia virus, reported earlier [10]. The compound with 4 pyrrole rings shows a higher inhibition than the one with 3 pyrrole rings. This correlation could not be well demonstrated with the compound containing 5 pyrrole rings. The compound Dist/5 is very unstable and longer incubations (90 min) may cause some degradation of this compound.

The inhibition of DNA polymerases of several on-

Table 4
Inhibition of DNA polymerase activity of MSV (M) by distamycin A and its derivatives in the presence of various templates*.

Antibiotic used**	^{3}H-TMP incorporation into DNA poly (dA–dT) (cpm/reaction mixt.)	^{3}H-dGMP incorporation into DNA poly (dI–dC) (cpm/reaction mixt.)	^{3}H-TMP incorporation into DNA poly rA–$(dT)_8$ (cpm/reaction mixt.)
None	624 (100)	570 (100)	412 (100)
Dist/2	204 (32.6)	459 (86)	328 (80)
Dist/A	144 (23)	441 (77)	280 (68.2)
Dist/5	60 (9.6)	447 (78)	214 (52)

* The incorporation in the absence of templates was 157 cpm/reaction mixt. for ^{3}H-TMP, and 61 cpm/reaction mixt. for ^{3}H-dGMP.

** Antibiotic concentration = 20 μg/reaction mixt. The figures in brackets indicate percent of the control. The reaction conditions are described under Materials and methods.

cogenic viruses by ethidium bromide using various templates has been recently reported [16]. This inhibition was found to be dependent on the nature of the template used and the source of enzyme. It was therefore of interest to study the distamycin inhibition of DNA-polymerase activity in the presence of various templates.

The effect of distamycin derivatives on the DNA-polymerase activity of FL-virions in the presence of poly (dA–dT), poly (dI–dC) and poly rA–$(dT)_8$ is shown in table 3. With poly (dA–dT) as primer-template, the DNA-polymerase activity was found to be most sensitive to distamycin inhibition. The system containing poly rA–$(dT)_8$ is also very sensitive to distamycin inhibition, though the response in this case is weaker than that observed in the presence of poly (dA–dT). It is important to note that in both cases the inhibitory response of the antibiotic increases according to the number of pyrrole rings in the molecule. With poly (dI–dC) as template no significant inhibition of DNA-polymerase activity by distamycin derivatives was observed.

Inhibition of DNA polymerase activity of MSV-M by distamycin A and its derivatives in the presence of various templates is shown in table 4. The DNA-polymerase activities catalyzed by poly (dA–dT) and poly rA–$(dT)_8$ are very sensitive to distamycin action. The range of inhibitions by individual derivatives is similar to that observed in FL-virions. However, the poly (dI-dC) dependent incorporation of dGMP by DNA polymerase was found to be more sensitive towards distamycins in this case.

The experiments reported here demonstrate that the distamycin inhibition of DNA polymerase activities of FLV and MSV-M are template specific. Templates containing thymine and adenine are highly sensitive to the action of distamycins. This inhibition is dependent on the number of pyrrole rings in the molecule, also observed in the transcriptase reaction earlier [10]. The inhibition of DNA polymerases of RNA oncogenic viruses and the foci formation by distamycin derivatives conclude that both activities are dependent on the same structural component(s) of the molecule. The *in vivo* activity of distamycin derivatives on the oncogenic response of these viruses will be reported elsewhere.

After this paper had been prepared for publication, we learned that Kotler and Becker [17] had studied the activity of Dist/A and Dist/5 on the reverse transcriptase activity of Rous sarcoma virions in the absence of exogenous templates. Their data agree with our results on FL-virions reported in table 2.

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