EFFECT OF DISTAMYCIN A AND CONGOCIDINE ON DNA SYNTHESIS BY ROUS SARCOMA VIRUS REVERSE TRANSCRIPTASE

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1. Introduction

Recent studies [1, 2] demonstrated that Distamycin A, a basic oligopeptide antibiotic produced by *Streptomyces distallicus,* inhibits the synthesis of DNA by Rous Sarcoma virus (RSV) reverse transcriptase [3–5]. It was found that Distamycin A and two of its derivatives, compounds II and XIII (fig. 1) have a partial inhibitory effect on the reverse transcriptase, even at the high concentrations tested (100 μg/ml). The inhibitory effect of the antibiotics can be explained by the ability of Distamycin A molecules to interact with double- and single-stranded DNA molecules [6, 7]. It was also reported that Distamycin A binds to polyguanylic acid [8] and to RNA [9]. In the present study we compared the effect of the antibiotic congocidine (fig. 1), which resembles Distamycin A, on the activity of RSV reverse transcriptase and the effect of Distamycin A on the nature of the DNA molecules synthesized *in vitro* by RSV enzyme.

2. Materials and methods

RSV, B_{77} strain, was harvested from the culture medium of transformed chick fibroblasts. The virus was concentrated 100-fold, purified by centrifugation in sucrose gradients (15–65%, w/v), treated with Nonidet P-40 and the enzymatic activity was assayed according to the method of Temin and Mizutani [3]. The assay system consisted of dATP, dGTP (Schwarz, Orangeburg, N.Y., USA) and dCTP (Sigma, St. Louis, Missouri, USA), 0.125 μmoles each in 0.02 M Tris-HCl buffer at pH 8.1, containing 0.33 mM EDTA and 1.7 mM dithiothreitol; 1.5 μmoles of $MgCl_2$ and 2.5 μmoles of KCl; 2.5 μg phosphoenolpyruvate (Sigma) and 10 μg pyruvate kinase (Sigma); 2.5 μCi of ^{3}H-TTP (Radiochemical Centre, Amersham, England; specific activity 10.35 Ci/mmole) and 0.025 ml of the virus preparation which contained 15–20 μg protein. The polymerase assays were carried out in the presence of various concentrations of the antibiotics. Distamycin A was obtained from Farmitalia, Italy and congocidine from Rhône-Poulenc Laboratories, France.

The nature of the DNA species synthesized by RSV reverse transcriptase in the absence or in the presence of Distamycin A and congocidine (100 μg/ml reaction mixture) was studied by elution from hydroxylapatite [10, 11] columns with a linear sodium phosphate gradient [10]. Hydroxyapatite was obtained from Bio-Rad Lab, Richmond, Calif. The reaction mixtures were dissolved with sodium dodecyl sulfate (1%, w/w, final concentration), loaded on the hydroxylapatite, eluted with a sodium phosphate gradient ranging from 0.05–0.4 M, collected into about 40 fractions and the TCA precipitable radioactivity was collected on filters and counted in a liquid scintillation counter. Herpes simplex virus (HSV) purified DNA, labeled with ^{14}C-thymidine was used as a marker (Gordin, Olshevsky and Becker, to be published). The HSV–DNA eluted from the hydroxylapatite column prior to HeLa cell DNA.

3. Results

The results presented in fig. 2 demonstrate that the antibiotics Distamycin A and congocidine affected the

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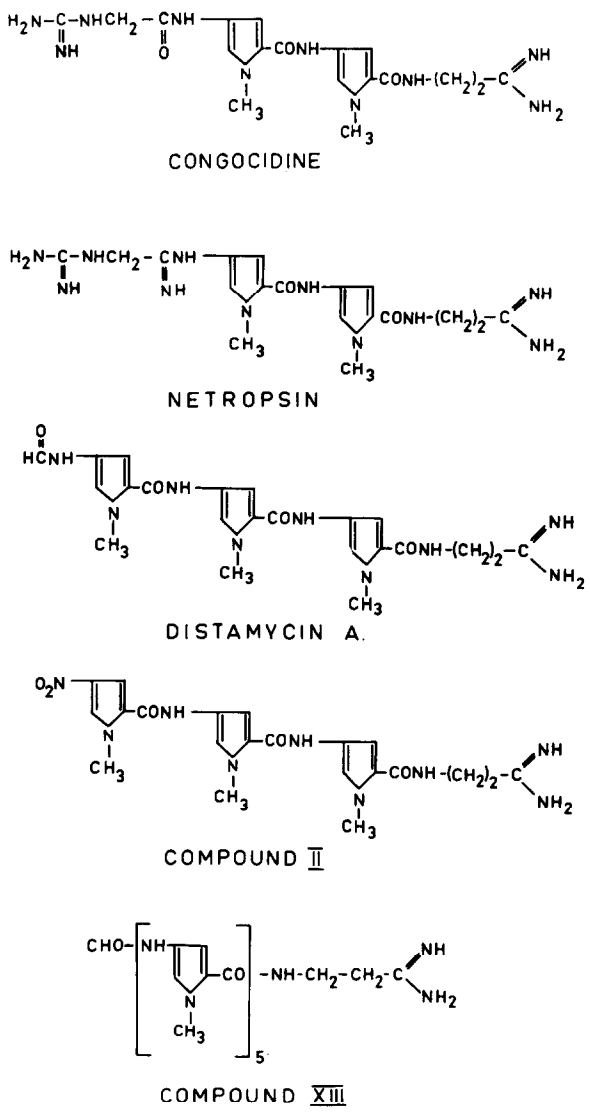

Fig. 1. The structure of Distamycin A and related compounds.

synthesis of DNA by RSV reverse transcriptase; the inhibitory effect of congocidine was less marked than that of Distamycin A. However, the extent of the inhibitory effect varied somewhat in the various experiments performed. In addition, it can be seen (fig. 2) that even at a concentration of 100 μg/ml Distamycin A only had a partial inhibitory effect on DNA synthesis by the RSV reverse transcriptase.

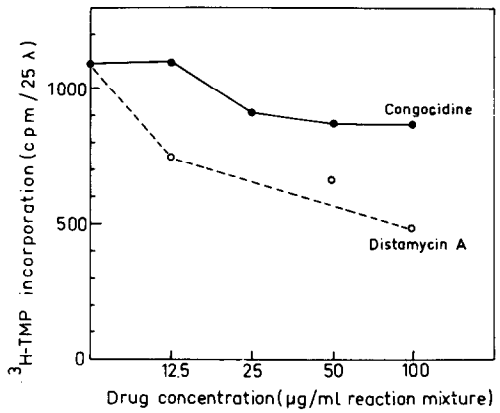

Fig. 2. The effect of Distamycin A and congocidine on DNA synthesis by RSV reverse transcriptase. The enzymatic reaction mixtures were treated with various concentrations of the antibiotics and incubated for 60 min at 37°. The radioactivity incorporated into the precipitable DNA was determined. The background of the reaction mixture, without the addition of the enzyme, is about 80–90 cpm.

The effect of Distamycin A (at a conc. of 100 μg/ml) on the kinetics of ^{3}H-TMP incorporation by the reverse transcriptase is shown in fig. 3. It was found that Distamycin A markedly affected, but did not prevent the synthesis of DNA by the RSV enzyme when added to the enzymatic reaction prior to the initiation of DNA synthesis. The ^{3}H-TMP incorporation in the presence of the antibiotic continued for 30 min and then stopped. The untreated enzyme continuously synthesized DNA for 60 min. The dilution of the enzyme with the buffer (fig. 3A) resulted in a reduction in the extent of ^{3}H-TMP incorporation. Addition of Distamycin A to the enzyme at 2 concentrations, 25 μg/ml and 100 μg/ml, 5 min after the initiation of DNA synthesis, also markedly affected the synthesis of DNA but ^{3}H-TMP incorporation continued for an additional period of 10 to 20 min. In this respect, the effect of Distamycin A on the RSV DNA polymerase differed from that of rifampicin [1], which completely inhibited DNA synthesis, but it resembled the inhibitory effect exerted by actinomycin D [2, 11].

The nature of the DNA products synthesized by the RSV reverse transcriptase in the presence of Distamycin A was further investigated (fig. 4). It was found that the viral DNA, which was synthesized by the untreated RSV reverse transcriptase *in vitro*

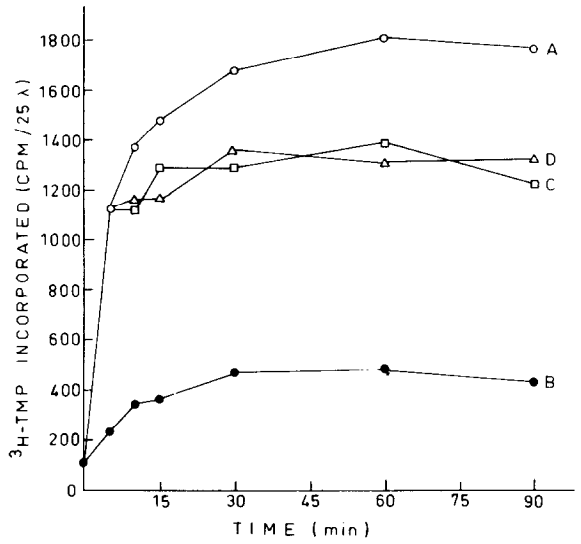

Fig. 3. Effect of Distamycin A on the kinetics of ^{3}H-TMP incorporation into DNA molecules by RSV DNA polymerases. Four polymerase assays (see fig. 1), each containing 250 μl were prepared. Distamycin A was dissolved in Tris-HCl EDTA buffer pH 8.1 and added to the reaction mixtures. One reaction mixture (A) served as a control, to (B) Distamycin A (final concentration 100 μg/ml) was added in the cold and the four tubes were incubated at 37°. At 5 min after initiation of DNA synthesis Distamycin A (25 μg/ml and 100 μg/ml) was added to tube (C) and (D), respectively. The same amount of buffer was added also to (A). At different time intervals, duplicate samples of 25 μl were removed from each reaction mixture and the TCA precipitable radioactivity was determined.

eluted as three species from a hydroxylapatite column. The first species to be eluted from the column contained single stranded DNA molecules (ssDNA). The second species contained the RNA:DNA hybrid molecules (hyDNA) and finally the double stranded DNA molecules (dsDNA) eluted just prior to the HSV-DNA marker (fig. 4A). Treatment of the DNA products with alkali (0.2 N, 18 hr at 37°) resulted in the denaturation of the double stranded DNA molecules and the appearance of ssDNA molecules which were eluted with the first DNA species (fig. 4A). Analysis of the DNA species synthesized by the reverse transcriptase in the presence of 100 μg/ml (2×10^{-4} M) Distamycin A (fig. 4B) demonstrated that ssDNA and hyDNA, but not dsDNA species, were synthesized. The antibiotic did not stimulate the synthesis of ssDNA and hyDNA. These results indicated that Distamycin A inhibited the enzymatic step necessary to form dsDNA from ssDNA and did not affect the synthesis of hyDNA and ssDNA. Analysis of the viral DNA species synthesized by RSV reverse transcriptase in the presence of congocidine revealed (fig. 4C) that although less ^{3}H-TMP was incorporated by the treated viral enzyme, all three DNA species were synthesized.

4. Discussion

Distamycin A was reported to inhibit the replication of DNA viruses [12, 13] and phages [14]. Studies on the physical properties of this antibiotic [6] demonstrated that the melting profile of native calf thymus DNA shifts towards higher temperatures when increasing antibiotic concentrations are added to the DNA solution. It was therefore concluded [6] that Distamycin A strongly binds to dsDNA and ssDNA, while less interactions have been demonstrated with ssRNA. Due to its ability to bind to DNA, Distamycin A also inhibits the template activity of both native and denatured DNA [6, 15]. These findings might also explain the effect of Distamycin A on DNA synthesis by RSV reverse transcriptase. We assume that Distamycin A has a low affinity to the RSV viral RNA and therefore does not inhibit the synthesis of hyDNA and ssDNA molecules. Due to its interaction with the viral ssDNA or hyDNA molecules, Distamycin A inhibits the synthesis of viral dsDNA.

The observation that congocidine had a lesser inhibitory effect on RSV reverse transcriptase than Distamycin A might indicate that the structure of the antibiotic molecule might influence its antiviral activity. The results presented here on the effect of congocidine can be compared to those reported on the effect of two derivatives of Distamycin A (compounds II and XIII, fig. 1) on RSV reverse transcriptase previously reported [1]. It can be concluded that a) an increase in the number of pyrrole rings increased the antiviral activity of the antibiotic. The most active compound XIII has 5 pyrrole rings; Distamycin A has 3 pyrrole rings; congocidine 2 pyrrole rings. b) The nature of the variable sidechain

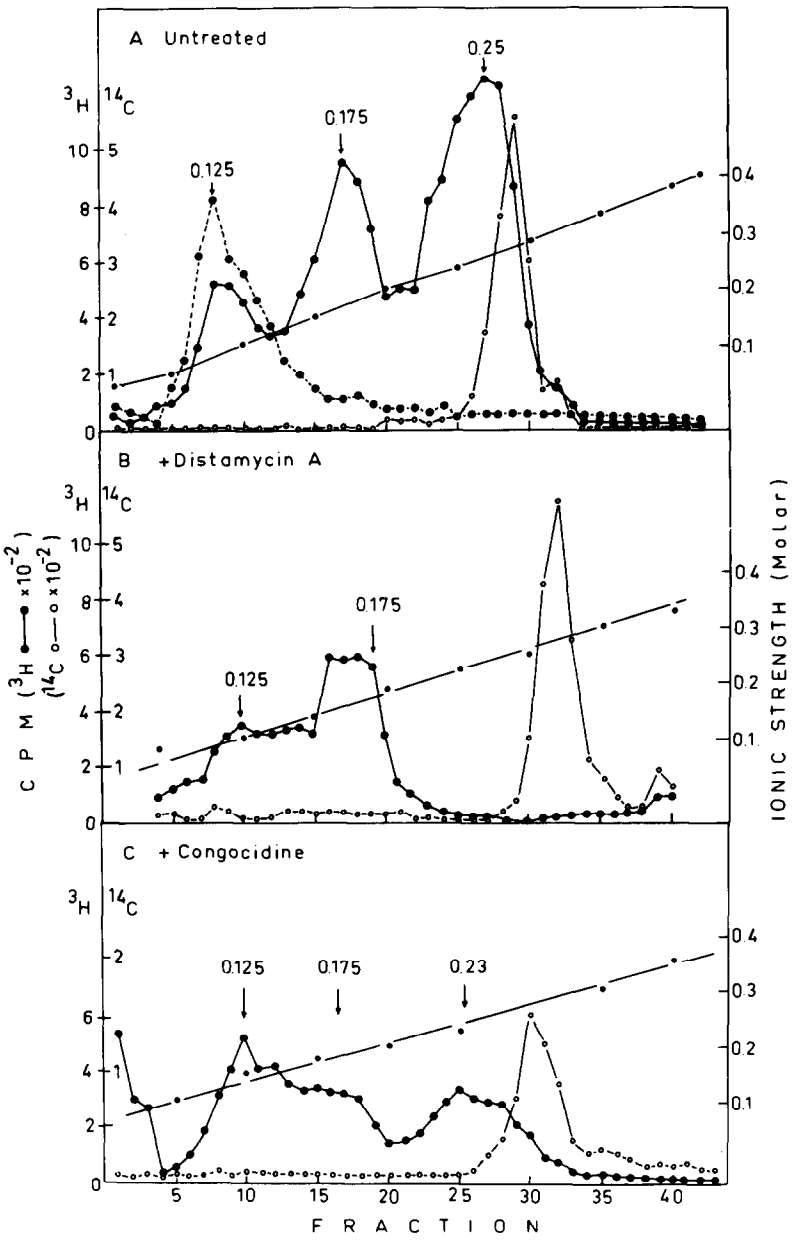

Fig. 4. Analysis of the DNA synthesized by RSV reverse transcriptase by elution from hydroxylapatite column. (A) The DNA molecules synthesized *in vitro* by B_{77} DNA polymerase. The dotted line represents a parallel experiment in which the reaction mixture was treated with 0.2 M NaOH for 18 hr at 37° and neutralized with 0.2 M HCl before loading on the column. (B) DNA molecules synthesized in the presence of 100 μg/ml Distamycin A and (C) DNA molecules synthesized in the presence of 100 μg/ml congocidine. Native Herpes Simplex Virus (HSV) DNA, labeled with ^{14}C-thymidine was used as a marker in each column. B_{77} virus reaction mixture (0.5 ml each; see text) was incubated for 60 min at 37° either without or with the antibiotics. Before analysis on the hydroxylapatite columns, sodium dodecyl sulfate (SDS) was added to each sample at a final concentration of 1% (w/v). Each column was filled with 1 g of hydroxylapatite (Bio-Rad Lab., Richmond, Calif.) and carefully washed with 0.05 M sodium phosphate. The columns were loaded with the reaction products together with HSV–DNA as a marker. The columns were washed with a solution of 0.05 M sodium phosphate buffer, pH 6.8, until equilibrium was reached. Macromolecules were eluted from the columns by a linear gradient of sodium phosphate, ranging from 0.05 M to 0.4 M. The effluent, total volume of 100 ml, was collected in 40 tubes, every 5th sample was measured for the refraction index by the Bausch and Lomb refractometer. The TCA precipitable radioactivity in each fraction was collected on filters and determined in a Packard liquid scintillation counter. (●—●—●) B_{77} virus product DNA; (○—○—○) HSV–DNA-^{14}C; (•—•—•) phosphate concentration; (●- -●- -●) alkali treated viral DNA.

has an additional importance in the antiviral activity as it was found that Distamycin A is more effective than its derivative compound II [1, 2]. This conclusion is in agreement with the findings of Chandra et al. [8] that the antiviral activity of Distamycins and their action on the template activity of DNA are dependent on the number of pyrrole rings and that the sidechain 1-methyl-pyrrole-2-carboxamide (present in Distamycin A and compound XIII) might be involved in the binding of the antibiotic to DNA [8].

The results of the present study indicate that Distamycin A, a natural fermentation product, whose structure was elucidated by total synthesis [16], might represent a group of antibiotics with activities against Oncorna virus reverse transcriptase. The requirement for pyrrole groups and sidechains might provide an approach to the synthesis of new antiviral substances with specific activities against viruses which have DNA genomes or DNA molecules as a primary transcription product.

Acknowledgements

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References

[1] M. Kotler and Y. Becker, Nature 234 (1971) 212.
[2] M. Kotler and Y. Becker, J. Israel Med. Ass. 81 (1971) 412.
[3] H.M. Temin and S. Mizutani, Nature 226 (1970) 1211.
[4] D. Baltimore, Nature 226 (1970) 1209.
[5] S. Spiegelman, A. Burny, M.B. Das, J. Keydar, I. Schlom, M. Travernicek and K. Watson, Nature 227 (1970) 563.
[6] P. Chandra, Ch. Zimmer and M. Thrum, FEBS Letters 7 (1970) 90.
[7] Ch. Zimmer, B. Puschendorf, H. Grunicke, P. Chandra and H. Venner, European J. Biochem. 21 (1971) 269.
[8] P. Chandra, A. Gotz, A. Wacker, M.A. Verini, A.M. Casazza, A. Fioretti, F. Arcamone and M. Ghione, FEBS Letters 16 (1971) 249.
[9] P. Chandra, A. Gotz, A. Wacker, M.A. Verini, A.M. Casazza, A. Fioretti, F. Arcamone and M. Ghione, FEBS Letters 19 (1972) 327.
[10] G. Bernardi, Biochim. Biophys. Acta 174 (1969) 423.
[11] J.P. McDonnell, A. Garapin, W.E. Levinson, N. Quintrall, L. Fanshier and J.M. Bishop, Nature 228 (1970) 433.
[12] M.A. Verini and M. Ghione, Chemotherapia 9 (1964) 144.
[13] J. Fournell, P. Ganter, F. Koening, Y. DeRatuld and G.H. Werner, Antimicrobial Agents and Chemotherapy (Williams and Wilkins, Baltimore, 1966) p. 599.
[14] A. DiMarco, M. Ghione, A. Sanfilippo and E. Morvillo, Experientia 19 (1963) 134.
[15] B. Puschendorf and H. Grunicke, FEBS Letters 4 (1969) 355.
[16] F. Arcamone, S. Penco, P. Orezzi, V. Nicolelia and A. Pirelli, Nature 203 (1964) 1064.