INFLUENCE OF TILORONE AND COGENERS ON THE SECONDARY STRUCTURE AND TEMPLATE ACTIVITY OF DNA

P. CHANDRA* and M. WOLTERSDORF

Gustav-Embden-Zentrum der Biologischen Chemie, Abteilung für Molekular-Biologie, Klinikum der J. W. Goethe-Universität, Frankfurt/M., Germany

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1. Introduction

The dihydro-chloride salt of 2,7-bis[2-(diethyl-amino) ethoxy]-fluoren-9-one, referred to as tilorone hydrochloride or bis-DEAE-fluorenone (DEAE-F), is a broad spectrum antiviral compound [1] with antitumor activity [2–4]. Tilorone hydrochloride has been shown by us to form a molecular complex with DNA [5]; specifically, with dAT-regions of the double-stranded DNA [6]. On the basis of our hydrodynamic studies with the tilorone–DNA complex we proposed an intercalative mode of binding of tilorone to DNA [6]. These interaction inhibit the DNA template functions in DNA- and RNA polymerase reactions in vitro [5]. Tilorone was also reported to inhibit the DNA polymerase activity in RNA tumor viruses [7]. The latter inhibition was found to be selectively dependent on the type of primertemplate used in the viral enzyme reaction.

Chandra et al. [8] have recently found that structural modifications in the tilorone molecule lead, in some cases, to a potentiation of its antiviral activity against MSV (Moloney). That, the oncogenic RNA viruses are capable of carrying out DNA synthesis, is now well estabilished. Thus, it was of interest to study whether, the interaction of tilorone with DNA and synthetic polydeoxynucleotides can be influenced by modifying tilorone structure. Such studies are indeed, important

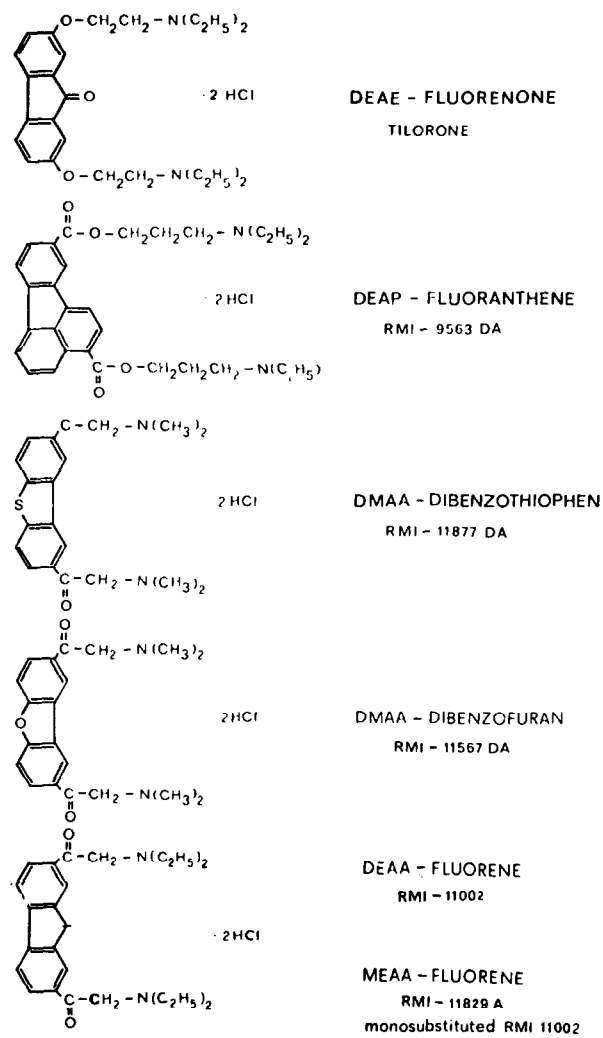

Fig. 1. Chemical structures of tilorone and cogeners.

* Correspondence should be sent to: Prof. Dr. P. Chandra, Gustav-Embden-Zentrum der Biologischen Chemie, Abteilung für Molekularbiologie, Klinikum der J. W. Goethe-Universität, Theodor-Stern-Kai 7, Frankfurt-Main 70, Germany.

to elucidate the role of structural entities in their complex formation with DNA. The cogeners used in the present study are shown in fig. 1.

2. Materials and methods

Radioactively labeled compounds were obtained from NEN-Chemicals GmbH, Germany; other triphosphates were supplied by Boehringer Mannheim GmbH, Tutzing, Germany. Calf thymus DNA was isolated according to the procedure of Zamenhof [9]. DNase (2000 Kunitz units per mg protein) was obtained from Serva, Heidelberg. *E. coli* K-12 cells were supplied frozen (mid-log phase) by Miles Chemical Labs., Elkhart, USA. Tilorone and cogeners were gift of Merrell-National Laboratories, Ohio, USA. All other chemicals were analytical grade reagents from Merck AG, Darmstadt, Germany.

2.1. *RNA polymerase reaction*

RNA polymerase was isolated from *E-coli* K-12 cells according to the procedure by Burgess [10] and kept in buffer containing 50% glycerol at −20°C. The reaction mixture contained, in 0.25 ml: 0.04 M Tris, pH 7.9; 0.01 M $MgCl_2$; 0.1 mM EDTA; 0.1 mM DTT; 0.15 mM UTP, CTP and GTP; 0.15 mM [^{3}H] ATP and 1 μg of the template; in experiments with poly (dA-dT), CTP and GTP were omitted from the reaction mixture. The reaction was started with 5–10 μg enzyme protein and incubations were carried out for 20 min at 37°C. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid (TCA) and serum albumin was used as carrier. The precipitate was collected on Whatman glass-fibre paper (GF/C) and washed 4 times with 3 ml of 2% TCA. The filter was dried and counted with toluol scintillation fluid in Isocap, Nuclear Chicago. Protein was estimated by the method of Lowry et al. [11].

2.2. *Physico-chemical measurements*

Spectrophotometric measurements were carried out on Zeiss PM QII spectrometer, equipped with a constand temperature bath.

3. Results and discussion

The effect of tilorone and cogeners (fig. 1) on the DNA-dependent RNA polymerase reaction is shown in fig. 2. The template activity of native DNA is strongly inhibited by DEAP-fluoranthene, showing an 80% inhibition at a concentration 8×10^{-6} M. Other derivatives, at this concentration do not show any significant inhibition of the template activity of DNA. However, at higher concentrations one observes a dose-dependent inhibition of DNA-template activity by DEAE-fluorenone, DMAA-dibenzothiophene, DEAA-fluorene and DMAA-dibenzofuran. The monoethyl derivative, MEAA-fluorene does not show any activity even, at higher concentrations.

It has been reported earlier [6] that tilorone pre-

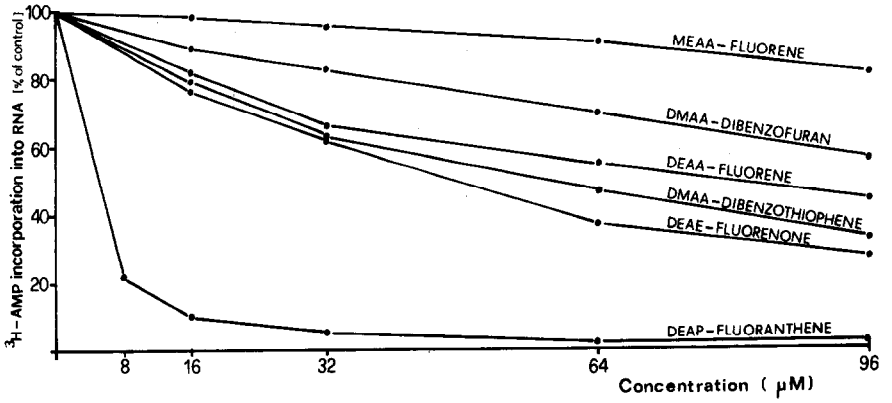

Fig. 2. Inhibition of DNA-dependent RNA polymerase reaction (*E. coli* K-12) by tilorone and cogeners. The reaction conditions are described under Materials and methods.

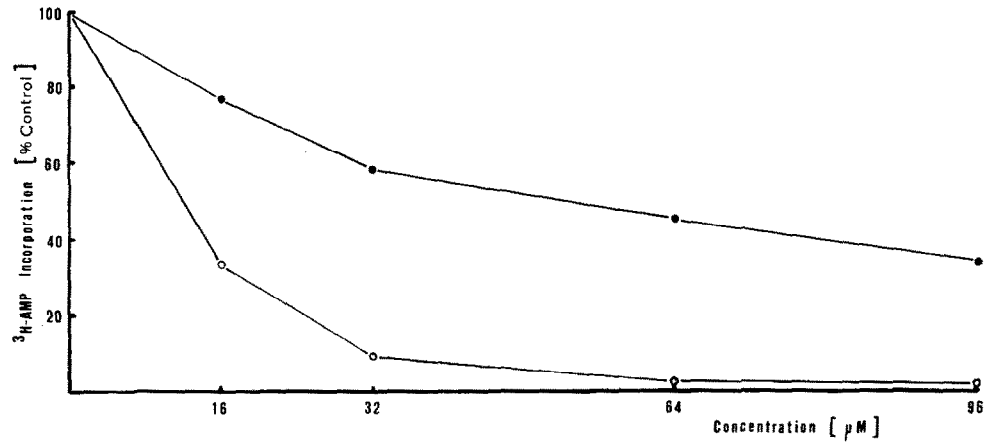

Fig. 3. Inhibition by tilorone of the RNA polymerase reaction catalyzed by the templates: Calf thymus DNA •–•–•; and poly (dA–dT) o——o. Regardless of the template, the reaction mixture (0.25 ml) contained 1 μg of the template. In the poly (dA–dT)-catalyzed reaction, GTP and CTP were omitted from the reaction mixture.

ferentially binds to the dAT regions of the DNA molecule. These studies were done by measuring the effect of tilorone on the melting (T_m) of DNA preparations from various sources, having A–T content 28%–65%. In the present study we could confirm the preferential binding of tilorone to dAT regions, using DNA and poly (dA–dT) in the RNA polymerase reaction (fig. 3). The template activity of poly (dA–dT) (o——o) in the RNA polymerase reaction is distinctly more sensitive towards tilorone than that of DNA (•——•); particularly, at low drug concentrations the poly (dA–dT)-catalyzed reaction is three times more sensitive than the DNA-catalyzed activity of RNA polymerase reaction.

The nature of the inhibitory response, as depicted in fig. 2 indicates that these compounds compete for the binding sites on DNA. This is clearly shown in the Lineweaver–Burk plot of the kinetic data obtained

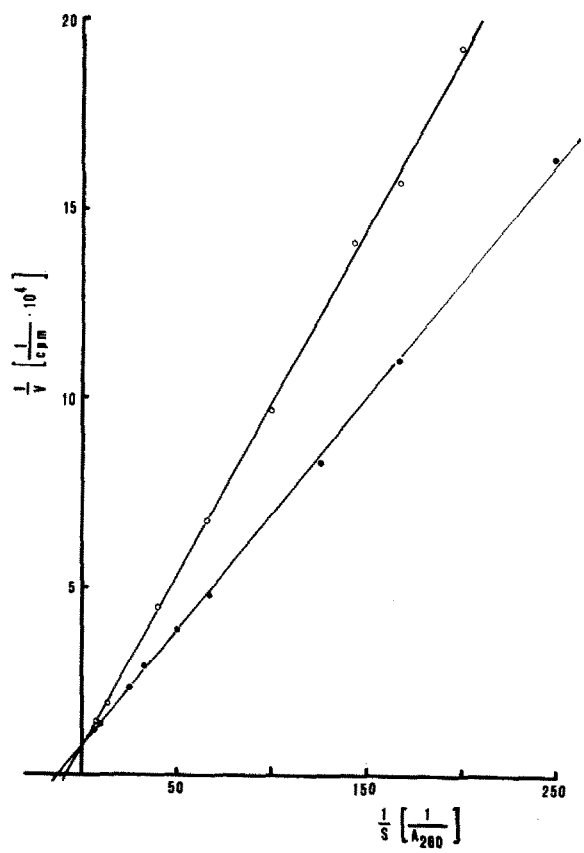

Fig. 4. Lineweaver–Burk plot of the effect of increasing concentrations of template DNA on the incorporation of [^{3}H] AMP into RNA by RNA polymerase of *E. coli* K-12, in the absence of tilorone (•——•), or in the presence of tilorone, 3.2 × 10^{-5} M (o——o). Abscissa: $1/S$ = (DNA template, A_{260} per reaction mixture)$^{-1}$. Ordinate: $1/V$ = (^{3}H-labelled AMP incorporated into RNA)$^{-1}$.

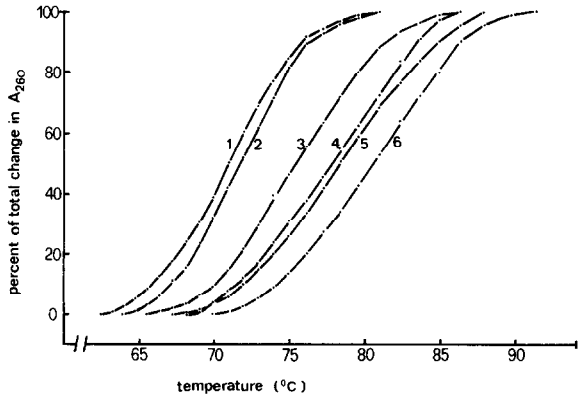

Fig. 5. Effect of tilorone and cogeners on the thermal transition temperature (T_m) of calf thymus DNA. Solvent is 0.01 M Tris–HCl pH 7.0, and the concentrations of DNA-P and cogeners are 5×10^{-5} M and 5×10^{-6} M, respectively. Curve 1 = DNA; 2 = DNA + MEAA-fluorene; 3 = DNA + DEAA-fluorene; 4 = DNA + DMAA-dibenzothiophene; 5 = DNA + DMAA-dibenzofuran and 6 = DNA + DEAE-fluorenone.

by measuring the RNA polymerase activity at various concentrations of template DNA (fig. 4). Fig. 4 depicts the kinetic curves of the reactions in the absence of the inhibitor (●——●), and in the presence of 3.2×10^{-5} M DEAE-fluorenone (○——○). This shows that DEAE-fluorenone is a competitive inhibitor of DNA template activity in the RNA polymerase reaction.

The inhibition of DNA template activity by tilorone and its cogeners is strongly influenced by substitutions in the ring (e.g. thiophene, furan etc.), as well as in the side chains. It was therefore, of interest to study whether, such substitutions influence their interaction to DNA. Fig. 5 depicts the melting curves of calf thymus DNA alone (curve 1), or in the presence of MEAA-fluorene (curve 2), DEAA-fluorene (curve 3), DMAA-dibenzothiophene (curve 4), DMAA-dibenzofuran (curve 5) and DEAE-fluorenone (curve 6). These studies were carried out at a drug/DNA-P ratio (r) of 0.1 Tilorone hydrochloride (DEAE-fluorenone) shows a large increase in the thermal transition temperature

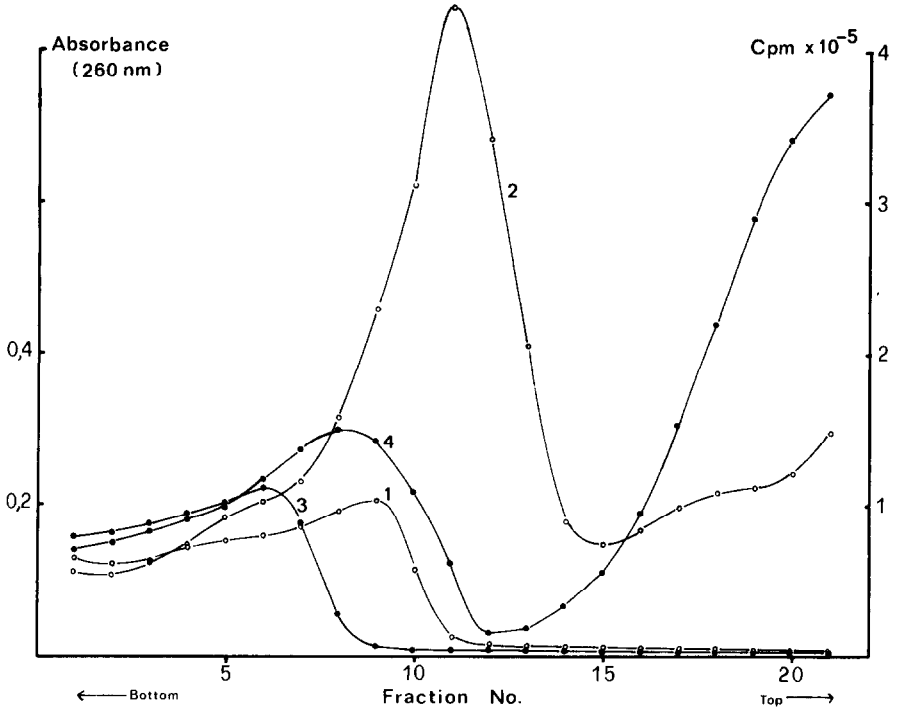

Fig. 6. Effect of Mg^{2+} on the binding of [9-^{14}C]tilorone hydrochloride to calf thymus DNA. Samples contained 2×10^{-4} M [9-^{14}C]tilorone hydrochloride and 9×10^{-4} M DNA-P (curve 2) in the presence of 10^{-2} M Mg^{2+} (curve 4) were layered on a linear sucrose gradient (5–20%) and centrifuged in SB 405 rotor (ICE ultracentrifuge) at 40 000 rpm for 15 hr. The gradients were analyzed in 12 drop fractions each. Curves 1 and 3 represent the optical densities of DNA without Mg^{2+} (curve 1), or in the presence of Mg^{2+} (curve 3).

(T_m) of native DNA (curve 6). The cogener DEAP-fluoranthene, under these conditions, showed a very similar response (curve not shown). It is interesting to note that DMAA-dibenzofuran though, less active than DMAA-dibenzothiophene and DEAA-fluorene in the RNA polymerase reaction, has a higher effect on the T_m of calf thymus DNA, than exhibited by these two derivatives.

The structure–activity relationship observed in the RNA polymerase reaction, is not strictly exhibited by the melting curves of DNA and cogener complexes. The latter studies were done in the absence of magnesium ions whereas, the RNA polymerase reaction requires magnesium ions for its enzymatic activity. The effect of magnesium ions on the tilorone binding to DNA is shown in fig. 6. The curves 1 and 3 depict the optical densities of DNA (9×10^{-4} M) alone, or in the presence of Mg^{2+} (1×10^{-2} M) respectively. The binding of ^{14}C-labelled tilorone (2×10^{-4} M) to DNA in the absence, or in the presence of Mg^{2+} is shown by curves 2 and 4 respectively. It is interesting to note that the presence of magnesium ions leads to an inhibition of tilorone binding to DNA. Our studies with other enzymatic systems have shown that tilorone chelates with divalent ions Mg^{2+} and Ca^{2+} (manuscript in preparation).

The fact that compounds of tilorone type form chelates with Mg^{2+} may explain differences in the structure–activity relationships of cogeners in the RNA polymerase reaction, and their effects on the melting behavior of DNA. Thus, the dibenzofuran cogener though, less active than DEAA-fluorene in the RNA polymerase reaction, has a higher affinity for DNA than DEAA-fluorene. The dibenzofuran derivative should have a higher tendency for chelating with magnesium ions, which leads to its partial inactivation in the RNA polymerase reaction.

Acknowledgement

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References

[1] Krueger, R. F. and Yoshimura, S. (1970) Federation Proc. 29, 635.
[2] Adamson, R. H. (1971) J. Natl. Cancer Inst. 46, 431.
[3] Munson, A. E., Munson, J. A. and Regelson, W. (1971) Intl. Colloq. on interferone and Interferone Inducers, Leuven, Belgium, Abstract No. 27.
[4] Munson, A. E., Munson, J. A., Regelson, W. and Wampler, G. L. (1972) Cancer Res. 32, 1397.
[5] Chandra, P., Zunino, F., Zaccara, A., Wacker, A. and Götz, A. (1972) FEBS Letters 23, 145.
[6] Chandra, P., Zunino, F., Gaur, V. P., Zaccara, A., Woltersdorf, M., Luoni, G. and Götz, A. (1972) FEBS Letters 28, 5.
[7] Chandra, P., Zunino, F. and Götz, A. (1972) FEBS Letters 22, 161.
[8] Chandra, P., Will, G. and Götz, A. Biochem. Pharmacol. (in press).
[9] Zamenhof, S. (1958) Biochemical Preparations 6, 8.
[10] Burgess, R. R. (1969) J. Biol. Chem. 244, 6160.
[11] Lowry, O. H., Rosebrough, N. I., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.