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DNA BINDING ACTIVITY AND INHIBITION OF DNA–PROTEIN INTERACTIONS

DIFFERENTIAL EFFECTS OF TETRA-*p*-AMIDINO-PHENOXYNEOPENTANE AND ITS 2′-BROMO DERIVATIVE

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Abstract—In the present study are reported the differential DNA binding activity of the anti-tumor polyamidine tetra-*p*-amidinophenoxyneopentane (TAPP-H) and its 2′-halo derivative (TAPP-Br), and their effects on the binding of the recombinant Epstein–Barr virus (EBV) nuclear antigen to a synthetic oligonucleotide mimicking the target DNA sequence present in the EBV genome. In addition, the proliferation kinetics and cell cycle analysis of human leukemia K562 cells treated with TAPP-H and TAPP-Br are reported. The possible *in vivo* relationship between DNA binding affinity and cytotoxicity is also discussed.

The aromatic polyamidine tetra-*p*-amidinophenoxy-neopentane (TAPP-H‖) and its 2′-halo derivative (TAPP-Br) have been described as strong inhibitors of the *in vitro* activity of a variety of serine-proteinases, including kallikrein, factor Xa, urokinase, trypsin and chimotrypsin [1–3]. Due to the well-established involvement of serine proteinases in a wide spectrum of biological processes, aromatic polyamidines have been proposed for the control of a number of diseases, such as inflammation, thrombosis and complement-dependent immune reactions [4, 5]. In addition, TAPP-H and TAPP-Br have been described by our research group as inhibiting both *in vitro* cell proliferation of tumor cell lines [6–8] and *in vivo* tumorigenicity of melanoma cells xenografted into nude mice [9]. With respect to these antitumor properties of tetra-amidines, we consistently found TAPP-Br to be more active than TAPP-H [8].

The observation that TAPP-H and related compounds display a strong structural analogy with other DNA binding drugs, such as berenil [10], prompted us to investigate: (a) possible binding of these aromatic polyamidines to DNA and (b) possible inhibition by these compounds of the interaction between nuclear transcriptional factors and target DNA sequences. With respect to this issue we [11, 12] and others [13] have demonstrated that DNA binding drugs, such as distamycin and related compounds, are powerful inhibitors of the binding of nuclear factors to synthetic oligonucleotides mimicking target DNA sequences.

The results presented in this paper demonstrate that both TAPP-H and TAPP-Br bind DNA, though to different extents. This differential activity is strongly in agreement with differential inhibitory effects on the binding of the Epstein–Barr virus (EBV) nuclear antigen (EBNA-1) to a target DNA sequence present in the EBV genome [14, 15].

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‖ Abbreviations: TAPP-H, tetra-*p*-amidinophenoxy-neopentane; TAPP-Br, 2′-bromo derivative of tetra-*p*-amidinophenoxyneopentane; EBNA-1, Epstein–Barr virus nuclear antigen 1; EBV, Epstein–Barr virus; mer, oligomer; EBNA-1-mer, double-stranded synthetic oligonucleotide containing the recognition site of EBNA-1; Oct-1, octamer binding protein 1; Oct-1-mer, double-stranded synthetic oligonucleotide containing the recognition site of Oct-1; ssDNA, salmon sperm DNA.

MATERIALS AND METHODS

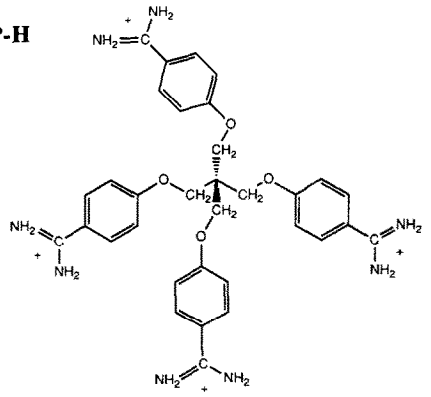
Synthesis of TAPP-H and TAPP-Br

The structures of 1,3-di-(*p*-amidinophenoxy)-2,2-bis-(*p*-amidinophenoxymethyl) propane (TAPP-H) and its bromo-derivative TAPP-Br are depicted in Fig. 1. TAPP-H and TAPP-Br were prepared by a minor modification of the procedure developed by Parsons [16]. Synthesis, melting points, yields, crystallization solvents and analytical data have been reported elsewhere [2].

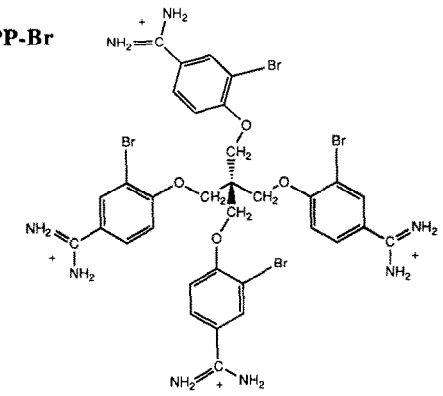
Cell lines and culture conditions

The human leukemia K562(S) cells used in these studies were grown in standard conditions in α-medium (Gibco, Grand Island, NY, U.S.A.), 50 mg/L streptomycin, 300 mg/L penicillin, supplemented with 10–15% fetal calf serum (Flow Laboratories, McLean, VA, U.S.A.) in 5% CO_2, 80% humidity, 37°. Cell growth was determined with a Model ZF Coulter Counter (Coulter Electronics, Hialeah, FL, U.S.A.).

A. TAPP-H



B. TAPP-Br



C. Berenil

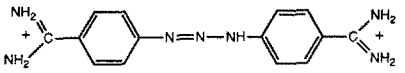


Fig. 1. Chemical structures of the aromatic polyamidines used in the present study: TAPP-H (A) and its bromo-derivative TAPP-Br (B). For comparison is reported the chemical structure of Berenil (C), another aromatic polyamidine which retains DNA binding properties [10].

Analysis of the cell cycle distribution of K562 cells

K562 cells were fixed in 70% ethanol and stored at −20° until analysis. The DNA content was measured using a Partec PAS II flow cytometer (Partec AG, Arlesheim, Switzerland) after staining with a dye solution containing equimolar (0.023 mM) concentrations of ethidium bromide (Serva, Heidelberg, West Germany) and mithramycin (Pfizer, New York, NY, U.S.A.) in 0.1 M Tris–HCl buffer (pH 7.6) with 15 mM $MgCl_2$ [17]. Excitation wavelength of around 405 nm of HBO 100 W mercury arc laser (Osram, Milan, Italy) was selected by a BG12 3 mm filter. A TK 455 dichroic mirror was used to separate the incident from the emission light. Red fluorescence was collected after a RG610 barrier filter. The flow cytometer was connected to a ND 620 multichannel analyser (Nuclear Data, Schamburg, IL, U.S.A.) and the signals accumulated on a 256 channel memory. DNA content distribution histograms were analysed by a specially designed software protocol running on a PC-XT IBM personal computer. The relative cell cycle fractions were derived according to the method proposed by Bertuzzi *et al.* [18]. A total of 2×10^4 events were accumulated for each histogram.

DNA binding activity of aromatic polyamidines

In order to determine the binding activity of the aromatic tetra-amidines TAPP-H and TAPP-Br the following experiments were performed.

Size exclusion chromatography. Sonicated salmon sperm DNA (ssDNA) (200 μg in 200 μL buffer) and the aromatic polyamidines (100 μL, 1 mM) were applied alone or in combination (after 15 min of incubation, to allow the complete binding of aromatic amidines to DNA) on a Sephadex G50 column (Pharmacia, Uppsala, Sweden; 1 cm diameter, 35 cm length), pre-equilibrated and eluted with isotonic Palitzsch buffer (pH 7.44) (for 100 mL of buffer solution, 10 mL of 0.05 M sodium tetraborate were mixed with 90 mL of 0.2 M boric acid; 270 mg of NaCl were added to adjust the tonicity of the buffer to 0.09 at 37°). The UV spectrum of each fraction was measured both at 260 and 280 nm in order to determine the concentration of DNA and TAPP. All the spectra were performed with a Jasco UVIDEC-510 double beam spectrophotometer.

DNA electrophoresis. The λ phage DNA fragments obtained by *Hind* III restriction enzyme digestion were electrophoresed in 1% agarose gel at constant voltage (100 mV) for 3 hr in the absence or presence of increasing concentrations (see figure legends) of TAPP-H and TAPP-Br. The relative band migrations were determined, after staining the gels with ethidium bromide and densitometric scanning.

Equilibrium dialysis. The test was continued by placing 3 mL of aqueous buffered solution containing 116 μg/mL TAPP-H (154 μM) and 165 μg/mL TAPP-Br (154 μM), or the same concentration of polyamidines plus 0.1 mg/mL ssDNA into a dialysis tube (M_r cut off 10,000–12,000; Medi Cell International, U.K.). The dialysis tubes were then placed into 90 mL of 0.1 M phosphate buffer, pH 7.4 under magnetic stirring. Samples of 200 μL were withdrawn at regular time intervals from 1 to 48 hr and analysed by reverse-phase HPLC for TAPP-H and TAPP-Br content.

Electrophoretic mobility shift assay

The electrophoretic mobility shift assay (i.e. gel retardation) was performed by using either the EBNA-1 recombinant protein or the crude extract from human leukemic K562 cells. The target oligonucleotides employed were the EBNA-1-mer and the Oct-1-mer (see Table 1), both purchased from Pharmacia. Binding reactions were set up in the following binding buffers: (a) 20 mM Tris–HCl, pH 7.6; 100 mM NaCl; 1 mM dithiothreitol; 10% glycerol; 0.05% NP-40; 5 mM $MgCl_2$, for EBNA-1-mer/EBNA-1 binding reactions; and (b) 20 mM Tris–HCl, pH 7.6; 50 mM KCl; 1 mM $MgCl_2$; 1 mM dithiothreitol; 0.01% Triton X-100; 5% glycerol; 0.5 mM spermidine, for Oct-1-mer/K562 cellular extract binding reactions, both in the presence of poly(dI:dC).poly(dI:dC) (Pharmacia). One microgram of either EBNA-1 factor or K562 nuclear extract and 0.25 ng of labeled double-stranded

Table 1. Sequence of EBNA-mer DNA

EBNA-mer	5'-AATTGAGCTCGGTACCCGGGGATCCTATCTGGGTAGCATATGCTAT- CTCGAGCCATGGGCCCCTAGGATAGACCCATCGTATACGATA- -CCTAATGGATCCTCTAGAGTCGACCTGCAGGCATGC-3' -GGATTACCTAGGAGATCTCAGCTGGACGTCCGTACGTTAA
Oct-mer	5'-AATTGCATGCCTGCAGGTCGACTCTAGAGGATCCATGCAAATGGA- CGTACGGACGTCCAGCTGAGATCTCCTAGGTACGTTTACCT- -TCCCCGGGTACCGAGCTC-3' -AGGGGCCCATGGCTCGAGTTAA

The binding site is underlined.

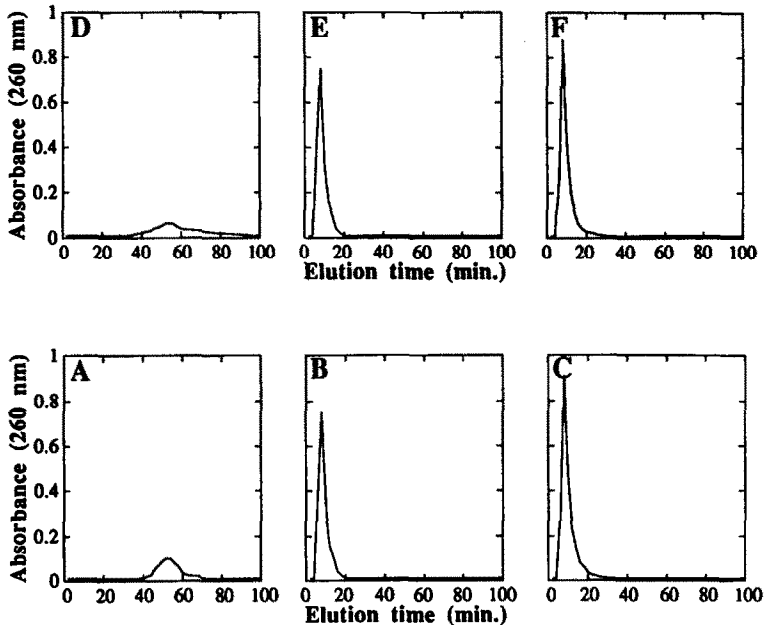


Fig. 2. Size-exclusion chromatographic profiles of free TAPP-H (A) and TAPP-Br (D), ssDNA (B, E), and ssDNA–TAPP-H (C) and ssDNA–TAPP-Br (F) complexes. Chromatographic runs C and F were performed after 15 min pre-incubation of ssDNA (200 μL, 1 μg/μL) with 100 μL of a 1 mM solution of the indicated polyamidine.

oligonucleotides (approximately 10,000 Cherenkow cpm) were in a total volume of 20 μL. After 30 min at room temperature, samples were electrophoresed at constant voltage (300 V for 2 hr) through a low ionic strength (30 mM Tris-borate buffer; 0.7 mM EDTA) on 6% polyacrylamide gels until tracking dye (Bromophenol blue) reached the end of a 16 cm slab. Gels were dried and exposed to X-Omat Kodak films at −80° with intensifying screens.

RESULTS

Binding of TAPP-H and TAPP-Br to DNA

Figure 1 shows the chemical structures of TAPP-H, TAPP-Br and berenil, another aromatic polyamidine retaining DNA binding activity [10]. Figure 2 shows the size-exclusion chromatography elution profiles (for experimental details, see Materials and Methods) of free TAPP-H and TAPP-Br (A and D, respectively), sonicated ssDNA (B, E), and ssDNA–TAPP-H (C) and ssDNA–TAPP-Br (F) complexes, produced after 15 min incubation of the aromatic polyamidines with DNA. As evident in this latter case, both TAPP-H and TAPP-Br co-elute with ssDNA (C, F), suggesting that these compounds bind DNA strongly under these experimental conditions.

In order to evaluate quantitatively the DNA binding activity of these drugs, we performed an analysis to determine firstly whether TAPP-H and TAPP-Br retard the electrophoretic migration of DNA fragments. The results obtained (shown in Figs 3 and 4) demonstrate that, in strong agreement with this hypothesis, both TAPP-H (Fig. 3) and TAPP-Br (Fig. 4) alter the electrophoretic mobility of *Hind* III fragments of bacteriophage λ DNA. Accordingly, we have found differential inhibitory effects of TAPP-H and TAPP-Br on the migration of double-stranded synthetic oligonucleotides and specific genomic fragments amplified by the

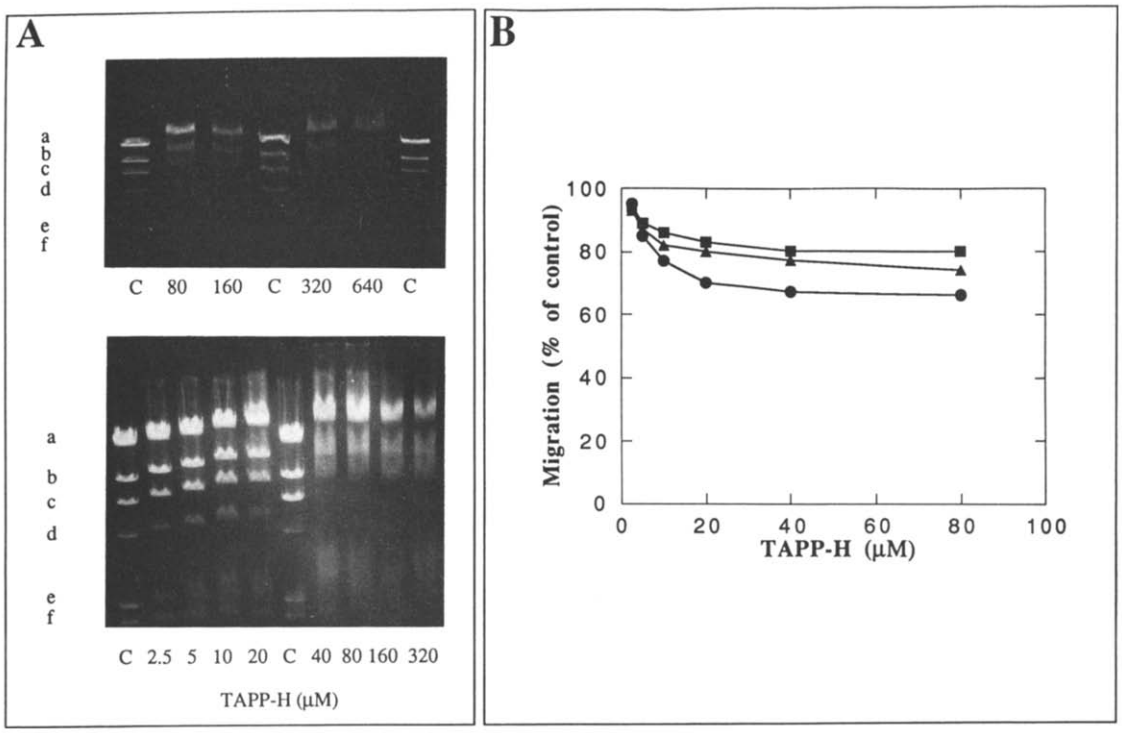


Fig. 3. (A) Effects of TAPP-H on the electrophoretic mobility of *Hind* III-generated fragments of λ phage DNA (a: 23.7 kb; b: 9.4 kb; c: 6.6 kb; d: 4.2 kb; e: 2.2 kb; f: 1.98 kb). Agarose gel (1%) electrophoresis of λ phage DNA was performed after 5 min preincubation with the indicated concentration of TAPP-H. C, untreated control DNA. (B) Quantitative analysis of the effects of TAPP-H on the electrophoretic migration of *Hind* III λ phage DNA fragments of different molecular masses. (■) 4.2 kb, (▲) 2.2 kb and (●) 1.98 kb.

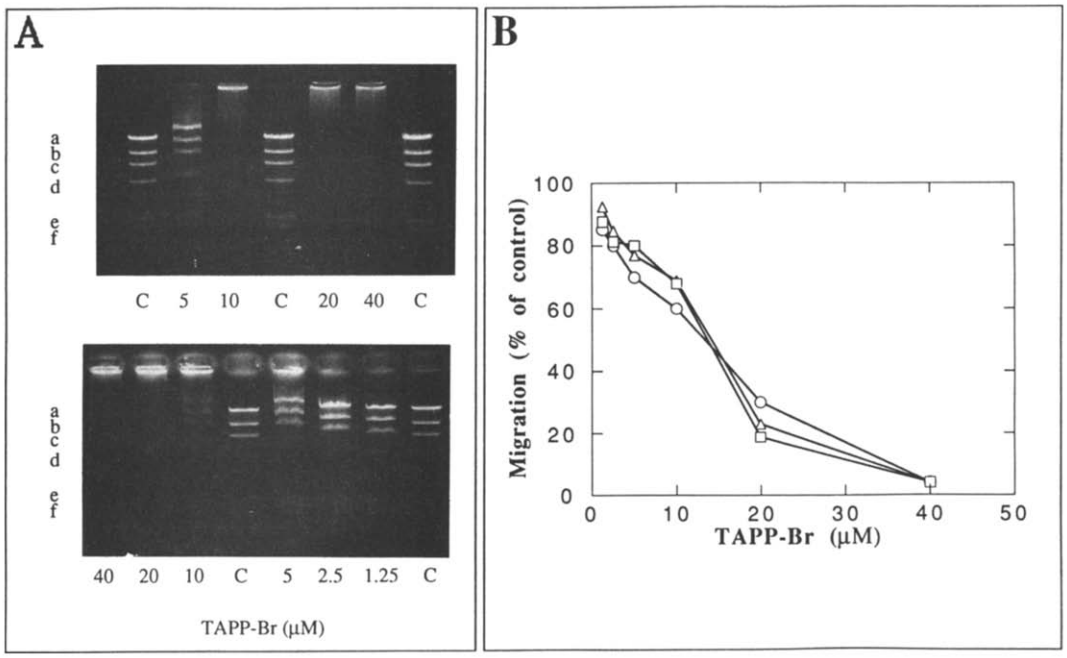


Fig. 4. (A) Effects of TAPP-Br on the electrophoretic mobility of *Hind* III-generated fragments of λ phage DNA (a: 23.7 kb, b: 9.4 kb; c: 6.6 kb; d: 4.2 kb; e: 2.2 kb; f: 1.98 kb). Agarose gel (1%) electrophoresis of λ phage DNA was performed after 5 min preincubation with the indicated concentration of TAPP-Br. C, untreated control DNA. (B) Quantitative analysis of the effects of TAPP-Br on the electrophoretic migration of *Hind* III λ phage DNA fragments of different molecular masses. (□) 4.2 kb, (△) 2.2 kb and (○) 1.98 kb.

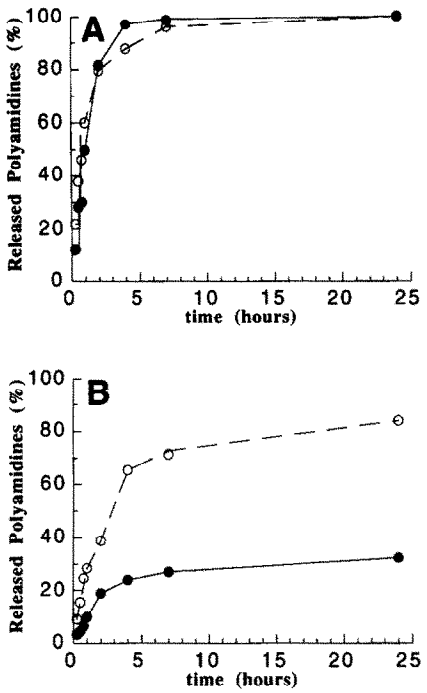


Fig. 5. Polyamidine release kinetics from dialysis tube determined for free compounds (A) or in the presence of 0.1 mg/mL of ssDNA (B). (○) TAPP-H; (●) TAPP-Br.

polymerase chain reaction (manuscript in preparation).

As deduced from the data shown in Figs 3 and 4, when 40 μM TAPP-H and 5 μM TAPP-Br are used, the electrophoretic migration of the 4.3 kb *Hind* III fragment of λ DNA is comparable to that of the 6.3 kb fragment, suggesting that about 2500 (TAPP-H) and 1800 (TAPP-Br) molecules of the compounds might interact with the 4.3 kb fragment. To investigate further whether TAPP-H and TAPP-Br might display different affinities to DNA, the experiment shown in Fig. 5 was performed. Aqueous solutions containing 116 μg/mL TAPP-H (154 μM) and 165 μg/mL TAPP-Br (154 μM) or the same concentration of polyamidines plus 0.1 mg/mL ssDNA were dialysed against a 30-fold excess of water. The TAPP-H and TAPP-Br release kinetics from the dialysis tube were determined by HPLC analysis. The results reported in Fig. 5A indicate that both TAPP-H and TAPP-Br are released within 4 hr with similar profiles. By contrast, TAPP-H and TAPP-Br show different release kinetics when the experiment is conducted in the presence of ssDNA. Both TAPP-H and TAPP-Br are released with kinetics slower than these exhibited by the free compounds. In addition, TAPP-Br is released with a much lower efficiency when compared to TAPP-H, suggesting that TAPP-Br displays higher affinity to DNA than TAPP-H. These data are in agreement with results published recently [19] showing that 2′-substituted polybenzamidines display a higher DNA binding affinity compared to the unsubstituted compounds.

Effects of TAPP-H and TAPP-Br on the binding of EBNA-1 to its target DNA fragment

When recombinant EBNA-1 protein is incubated with its target synthetic oligonucleotide (reported in Table 1) in the presence of different concentrations of TAPP-H or TAPP-Br, it appears evident that its binding activity decreases (Fig. 6A and C). Figure 6B shows that the retarded band generated by the interaction between EBNA-1 and the synthetic oligonucleotide carrying its target sequence is sharply different from the retarded band generated by the interaction between the crude nuclear extracts from K562 cells and the Oct-mer oligonucleotide containing the octamer 5′-ATGCAAAT-3′ motif. The specificity of the binding of EBNA-1 to the EBNA-mer is further demonstrated by the competition experiments performed utilizing both cold Oct-mer and the Sp-1-mer oligonucleotides, whose addition to the binding reaction did not affect the interaction of the EBNA-1 protein with the target sequence (data not shown).

In order to quantitate the effects shown in Fig. 6A, densitometric analysis of the autoradiograms was performed, and the binding activities of EBNA-1 obtained in the presence of TAPP-H and TAPP-Br were compared to that displayed by EBNA-1 in the absence of the aromatic tetra-amidines. The results depicted in Fig. 5C show that 40–60 μM TAPP-H and 5–10 μM TAPP-Br are the concentration ranges needed to obtain a 50% inhibition of the binding of EBNA-1 to the EBNA-mer oligosequence.

Inhibition of proliferation of K562 cells by treatment with TAPP-H and TAPP-Br

The inhibition of human leukemic K562 cell proliferation by TAPP-H is demonstrated by the results reported in Fig. 7. Fifty per cent inhibition of cell growth is obtained when 25–50 μM TAPP-H are added to the culture medium. In addition, the results shown in Fig. 7 indicate that the halo derivative TAPP-Br is more active than TAPP-H (50% inhibition of cell growth is in this case obtained with 2–5 μM TAPP-Br).

The same differential effects of TAPP-H and TAPP-Br on tumor cell growth were reproduced when the experiments were performed on other tumor cell lines, including erythroleukemic (FLC, K562 and HEL), B-lymphoid (Wl-L2), breast carcinoma (MCF7 and T47D), kidney carcinoma (KJ29) and melanoma (Colo 38 and M10) cell lines (Table 2).

Effects of TAPP-H and TAPP-Br on the distribution of K562 cells through the cell cycle

The results of flow cytometric analyses of K562 cells treated for 3 days with 5, 10, 30 and 50 μM TAPP-H or TAPP-Br are shown in Fig. 8. The quantitative data from this experiment are shown in Table 3. No significant alteration of the distribution of K562 cells through the cell cycle was detected following treatment with TAPP-H at any concentration and with 5–15 μM TAPP-Br. It should be

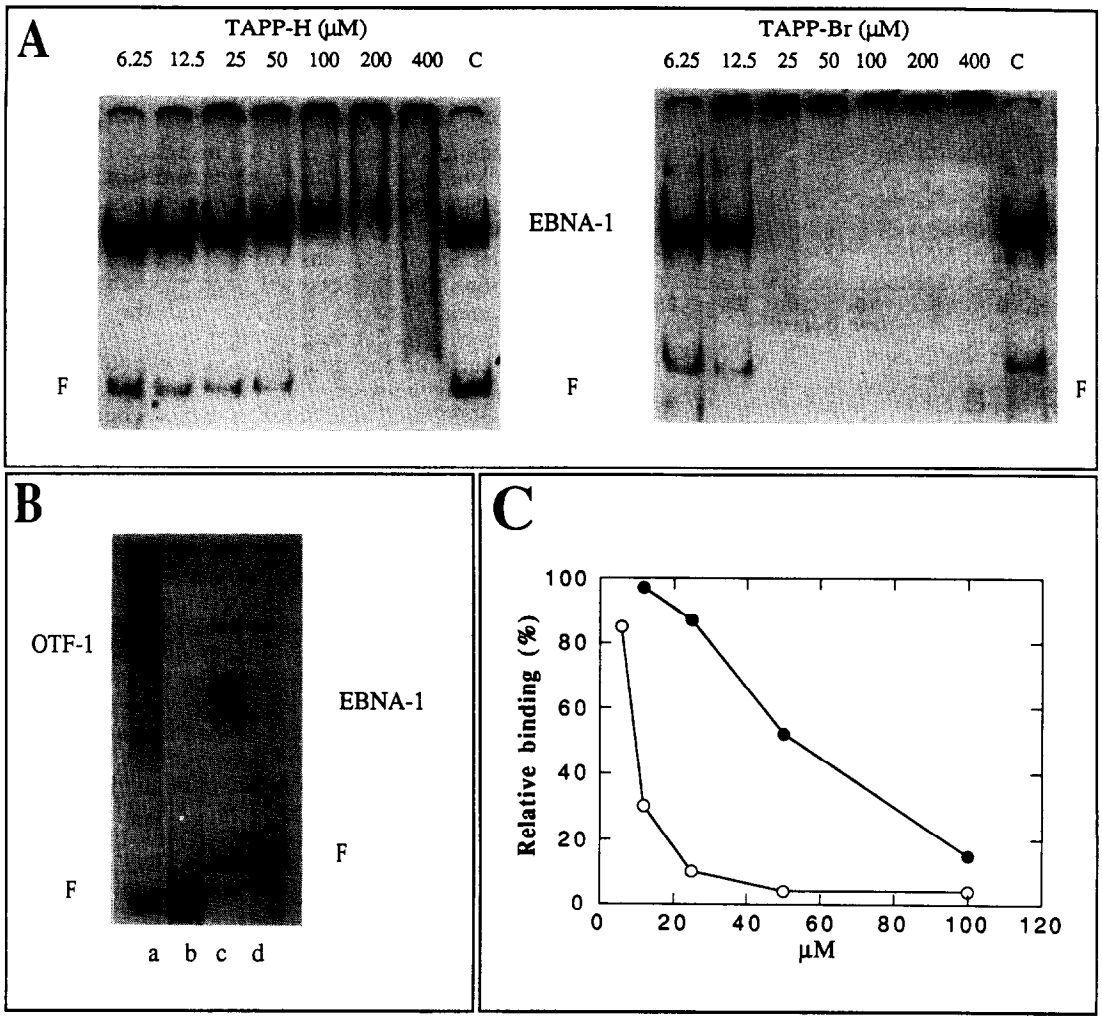


Fig. 6. (A) Effects of TAPP-H and TAPP-Br on the binding of EBNA-1 to a double-stranded oligonucleotide mimicking its target DNA sequence. The indicated amounts of polyamidine were added to ^{32}P-labeled EBNA-mer. After 30 min at room temperature the reaction mixtures were electrophoresed on 6% polyacrylamide gels. (B) Patterns of gel retardation obtained after incubation of the K562 nuclear extracts (a) and EBNA-1 (c) with Oct-mer and EBNA-mer ^{32}P-labeled oligonucleotides, respectively. Electrophoretic migrations of the Oct-mer (b) and EBNA-mer (d) are shown. (C) Densitometric analysis of the effects of TAPP-H (●) and TAPP-Br (○) on the specific EBNA-1 retarded band. F, free oligonucleotides.

underlined that inhibition of cell growth is detectable even when K562 cells are treated with 25–50 μM TAPP-H or 5–15 μM TAPP-Br (Fig. 7), in the absence of alterations in the distribution of the cells in the G_1, S and G_2 phases of the cell cycle (Fig. 8). Cell viability assessed by Trypan blue exclusion was found to be always higher than 95% in K562 cells treated for 3 days with 5, 10, 30 or 50 μM TAPP-H, and 5 or 10 μM TAPP-Br. Lower viability of 65–70 and 40–50% was found in K562 cells treated with 30 and 50 μM TAPP-Br, respectively.

These results suggest that at 25–50 μM TAPP-H or 5–50 μM TAPP-Br a lengthening of all the phases of the cell cycle is obtained. By contrast, when K562 cells are treated with 50 μM TAPP-Br, a sharp decrease in the proportion of S phase cells was observed associated with an increase in the proportion of G_2 phase cells, suggesting that TAPP-Br is more active than TAPP-H in inducing alterations in the cell cycle.

DISCUSSION

Results published recently by a number of laboratories, including ours, consistently indicated that the aromatic polyamidine TAPP-H and its halo derivative TAPP-Br are powerful inhibitors of serine proteinases, including trypsin, kallikrein, chimotrypsin and factor X [1–3]. Therefore, these and other synthetic proteinase inhibitors have been

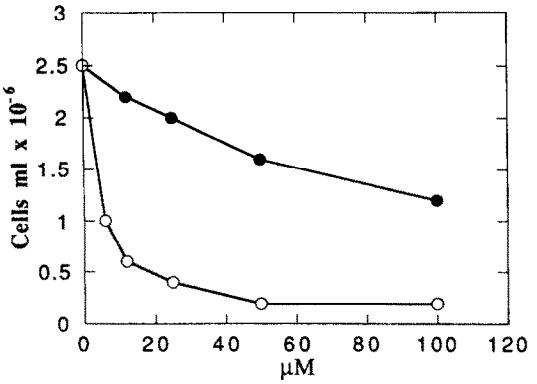


Fig. 7. Effects of TAPP-H (●) and TAPP-Br (○) on the *in vitro* proliferation of K562 erythroleukemic cells. Determinations were performed after 6 days of cell culture.

Table 2. Antiproliferative activity of TAPP-H and TAPP-Br

Tumor cell line	Concentration of polyamidine required for 50% growth inhibition	
	TAPP-H	TAPP-Br
FLC (erythroleukemic)	8	3
K562 (erythroleukemic)	67	8
HEL (erythroleukemic)	45	6.5
WI-L2 (lymphoid)	47	8.5
MCF7 (breast carcinoma)	62	10
T47D (breast carcinoma)	50	9
Colo 38 (melanoma)	42	6
M10 (melanoma)	10	3
KJ29 (kidney carcinoma)	91	18

Determinations were performed after 4 days of cell growth.

The results represent the mean values of three independent experiments.

proposed for the experimental treatment of a number of human pathologies, notably pulmonary enphysema [4] and cancer [6, 7]. It is well known, indeed, that serine proteinases play a direct role in a number of different physiopathological processes such as the activation of zymogens in the coagulation cascade [20]. With respect to the effects of serine proteinases on cell proliferation and invasion, it should be noted that: (a) proteinase-mediated insulin-independent activation of insulin receptor could potentiate *in vitro* tumor cell growth [21] and (b) the conversion of type IV pro-collagenase to active form (enzyme involved in the metastatic process) depends on proteinase activity [22].

In addition to the described effects on serine proteinases [1–3], aromatic polyamidines could exert their antitumor activity through alternative mechanism(s) of action. The main conclusion gathered from the results presented in this paper is indeed that both TAPP-H and TAPP-Br bind DNA and interfere with the binding of nuclear proteins to their specific target DNA sequences. These effects are in the case of TAPP-H comparable to those obtained with distamycin [12], a DNA binding drug which does bind selectively to AT-containing DNA elements and is able to suppress DNA–protein binding [13]. Our data demonstrate that TAPP-Br is more active than TAPP-H in inhibiting the interaction between nuclear factors and target DNA sequences. The differential affinity to DNA exhibited by TAPP-H and TAPP-Br might contribute to the differential inhibitory activity of these compounds on DNA–protein interaction, as suggested by the results presented in Figs 3, 4 and 5. However, it should be pointed out that our data do not formally exclude the possibility that TAPP-H and TAPP-Br bind EBNA-1 under the band-shift experimental conditions.

In conclusion, despite the fact that our data do not demonstrate that TAPP-H and TAPP-Br bind DNA in intact cells, they suggest that this is at least one of the mechanisms accounting for the strong antiproliferative effects of these compounds on both normal and tumorigenic cells. Fully in agreement with this hypothesis, we found that TAPP-Br is more active than TAPP-H in inhibiting cell growth and in inducing alterations in cell cycle parameters (Figs 7 and 8). The gel retardation approach described in the present paper could represent a rational approach to testing the activity of potential anti-tumor and antiviral drugs on both eukaryotic and viral genes.

Concerning the biological effects of the aromatic polyamidines, we hypothesize that TAPP-H and TAPP-Br could inhibit gene transcription by altering the activity of transacting factors. We would like to underline this point since the binding of transfactors to specific DNA sequences is an essential prerequisite to activate transcription [23]. Indeed, it is well established that the frequency of initiation of transcription by RNA polymerase II depends on proteins interacting with regulatory gene regions, leading to tissue-specific, developmentally regulated expression of many eukaryotic genes [24]. In addition, interaction between transcriptional factors (for instance CF-1, Sp1, IRBP, PuF, SIF) and oncogene promoters (jun, c-myc, c-Ha-ras) plays an important role in carcinogenesis [24, 25]. Furthermore, the interaction between cellular nuclear factors and retroviral long terminally repeated units is important in the transcriptional activation of retroviral genomes (for instance HIV-1) [23]. Accordingly, the results presented in this paper appear to be of some interest, as they suggest that TAPP and related drugs could be proposed as antiviral agents. On the other hand, we like to point out that, as for other DNA binding drugs (such as distamycin and daunomycin [13], TAPP treatment of experimental animals could lead to long term side effects (such as neoplastic transformation). It is well known, indeed, that activation of cellular oncogenes could be due to pharmacological mediated inhibition of the binding of negative transcriptional factors to specific DNA sequences [25]. Therefore, our data introduce serious cautions to the use of polyamidines in the experimental therapy of disease processes,

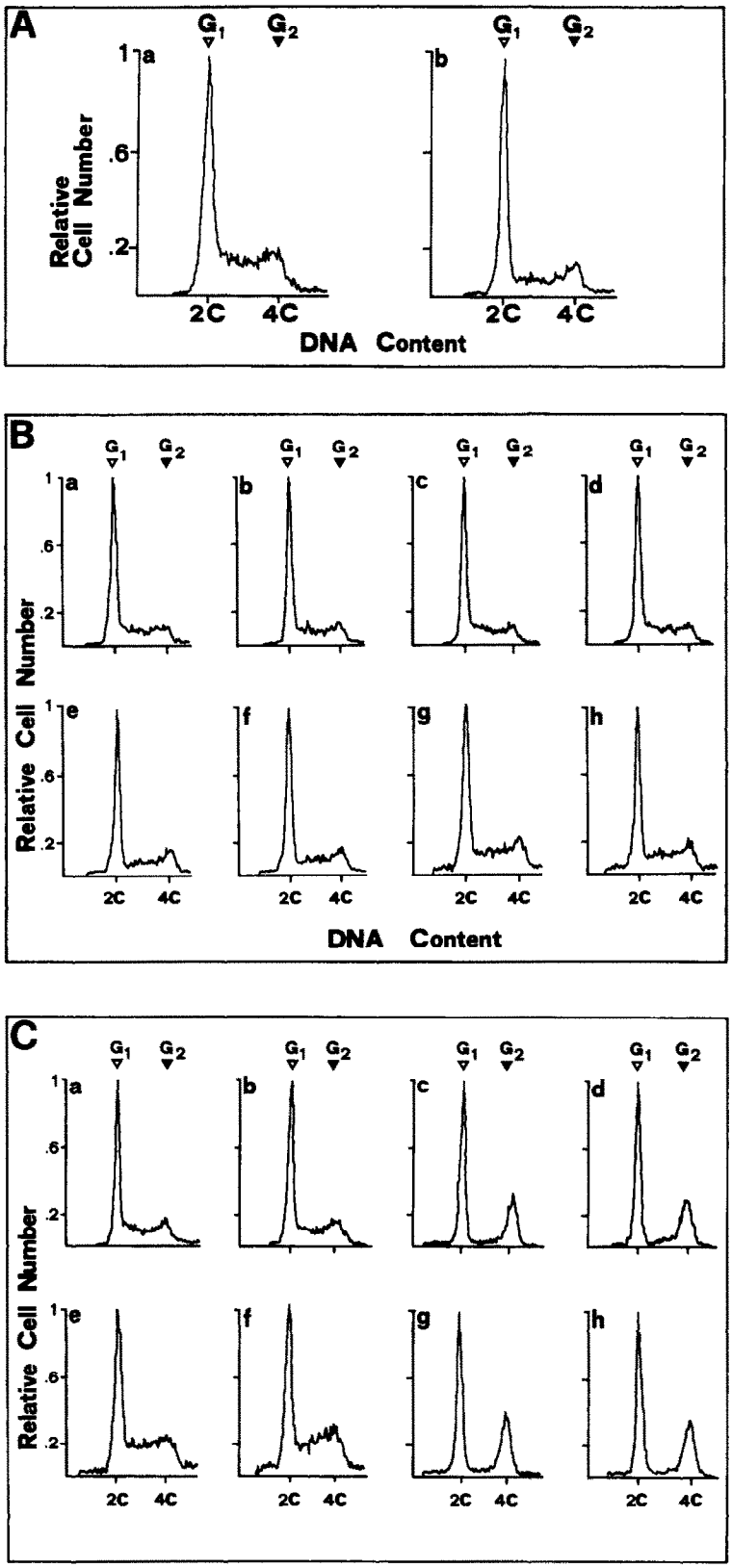


Fig. 8. Analysis of the effects of TAPP-H (B) and TAPP-Br (C) on the cell cycle distribution of K562 cells. Cells were cultured for 3 (a–d) or 6 (e–h) days in the presence of 5 (a, e), 10 (b, f), 15 (c, g) and 30 (d, h) μM of the indicated polyamidines. (A) Cell cycle distributions of control K562 cells cultured for 3 and 6 days.

Table 3. Effects of TAPP-H and TAPP-Br on the cell cycle of K562 cells

Cell cycle phase				
G1	S	G2+M	Days of cell culture	Concentration (μM)
Control				
46.4	45.4	8.2	3	
55.5	36.6	7.9	6	
TAPP-H				
57.3	36.2	6.5	3	5
53.4	37.1	9.5	6	5
55.7	36.1	8.2	3	10
53.3	40.0	6.7	6	10
54.0	42.0	4.0	3	30
48.0	43.8	8.2	6	30
56.5	39.9	3.6	3	50
48.7	43.9	7.4	6	50
TAPP-Br				
48.7	42.7	8.6	3	5
39.6	51.2	9.2	6	5
50.6	38.4	11.0	3	10
36.0	53.1	10.9	6	10
56.5	13.9	29.7	3	30
52.4	13.5	34.1	6	30
53.4	16.1	30.6	3	50
53.7	16.6	32.7	6	50

Results are expressed as proportion (%) of K562 cells at the indicated phases of the cell cycle. M, mitosis.

such as those occurring during inflammation, thrombosis and complement-dependent immune reactions [4, 5], due to their DNA binding properties.

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