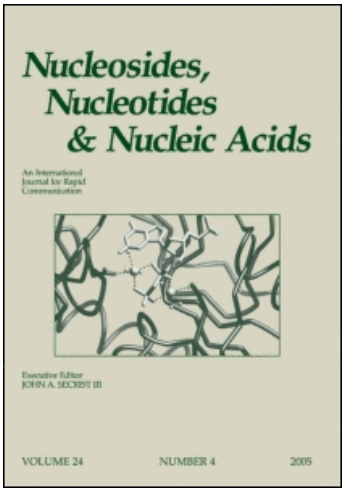

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Binding of G-Quadruplex-interactive Agents to Distinct G-Quadruplexes Induces Different Biological Effects in MiaPaCa Cells

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BINDING OF G-QUADRUPLEX-INTERACTIVE AGENTS TO DISTINCT G-QUADRUPLEXES INDUCES DIFFERENT BIOLOGICAL EFFECTS IN MiaPaCa CELLS

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□ *Our previous studies have demonstrated the preference of telomestatin for intramolecular, rather than the intermolecular, G-quadruplex structures, while TMPyP4 has selectivity for intermolecular over intramolecular G-quadruplex structures. However, it was not clear whether the difference in the selectivity between two different G-quadruplex-interactive agents could determine the corresponding biological effects in cultured human tumor cells. Here we evaluated the biological effects of both TMPyP4 and telomestatin in the human pancreatic carcinoma cell line (MiaPaCa) using subtoxic and cytotoxic concentrations. The cytotoxicity of these agents against MiaPaCa cells is quite different, and the IC_{50} of telomestatin (0.5 μM) is about 100 times less than that of TMPyP4 (50 μM). At IC_{50} concentrations, TMPyP4 induced anaphase bridge formation in MiaPaCa cells, while telomestatin failed to induce anaphase bridge formation. At subtoxic concentrations, TMPyP4 induced MiaPaCa cell growth arrest, senescence, apoptosis, and telomere length shortening within 5 weeks, while similar biological effects were evident after 12 weeks following treatment with telomestatin. Our data suggest that binding of G-quadruplex-interactive agents to distinct G-quadruplexes could induce different biological effects in human cancer cells.*

Keywords G-Quadruplex-interactive agent; TMPyP4; Telomestatin; Telomere

In honor and celebration of the life and career of John A. Montgomery.

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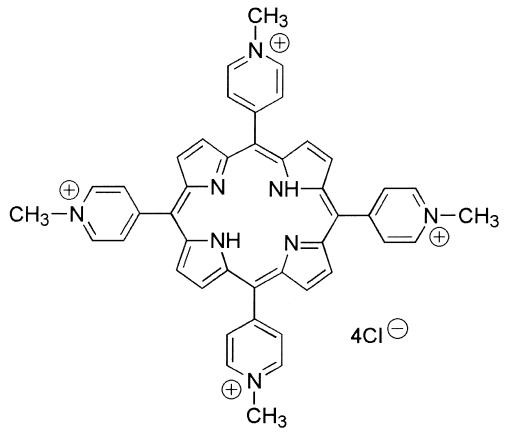
INTRODUCTION

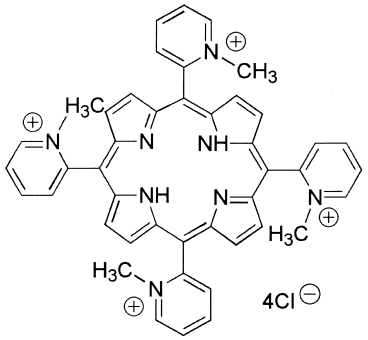
The telomeres at the ends of eukaryotic chromosomes are unique nucleoprotein structures, consisting of telomeric DNA and proteins that bind specifically to these sequences.[1,2] Telomeres are essential for maintaining genomic stability by providing protective caps at the ends of chromosomes.[1,2] Telomeric DNA in all vertebrates consists of tandem repeats of the hexanucleotide sequence 5′-TTAGGG-3′.[1,2] Telomeric DNA in most human somatic cells becomes progressively shortened due to the end-replication problem.[3] Thus, the cumulative loss of telomeric DNA after many cell divisions results in a limited replicative capacity.[3–5] This process is proposed to function as a biological clock, eventually limiting the proliferative life span of somatic cells and leading to cellular senescence.[3–5] However, some cell types, such as germ line or most cancer cells, can overcome this end-replication problem through the reactivation of telomerase, which replenishes telomeric DNA at the ends of chromosomal DNA, obtaining an unlimited replicative capacity.[6–8] Telomerase is a unique ribonucleoenzyme that consists of RNA and protein components, and a short RNA motif serves as a template for the synthesis of telomeric DNA.[6–8] Since telomerase expression is specific to cancers, telomerase would be an ideal target for anticancer drug development.[9,10] On the basis of the recently accumulated knowledge on various aspects of human telomerase function and biology, including composition, assembly, and enzymatic properties, several different strategies have been tried to achieve efficient telomerase inhibition.[9–12]

G-rich sequences have been reported to form unique G-quadruplex structures consisting of two or more G-tetrads in the presence of monovalent cations such as Na^+ and K^+.[13,14] In particular, the single-stranded form of a G-rich sequence of telomeric DNA is capable of forming intramolecular antiparallel basket G-quadruplexes in the presence of Na^+ and intramolecular parallel G-quadruplexes in the presence of K^+.[15,16] It has been proposed that small organic molecules that stabilize or induce G-quadruplex structures are likely to inhibit telomerase activity by sequestration of the substrate required for this activity, although the biological effects of these molecules may be more directly related to telomere disruption.[11,17] In previous studies, several groups of compounds, including anthraquinone analogues, porphyrins, perylenes, 9-anilino proflavine, triazine, pentacyclic acridine, fluoroquinophenoxazines, and telomestatin, have been reported to interact with G-quadruplexes.[17–26] Some of these compounds are among the most potent small-molecule inhibitors of telomerase reported to date.[25] Interestingly, several studies using these G-quadruplex-interactive compounds have shown encouraging data beyond telomerase inhibition, including telomeric disruption and short-term biological effects such as formation of anaphase bridges, apoptosis, and in vivo activity in mouse xenograft models.[17,26–29]

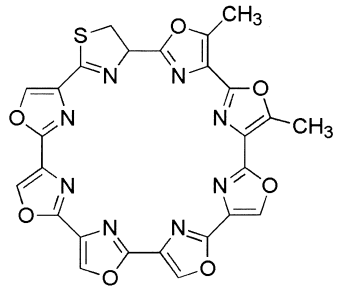
Our previous studies demonstrated the difference in the selectivity between the G-quadruplex-interactive agents telomestatin and TMPyP4 (Figure 1).[27,30] Telomestatin shows preferential binding for intramolecular over intermolecular G-quadruplex structures, while TMPyP4 has selectivity for intermolecular over intramolecular G-quadruplex structures.[27,30] The results from these studies also suggested that the difference in the selectivity between two different G-quadruplex-interactive agents could determine the corresponding biological effects of these compounds, including telomerase inhibition, telomere erosion, and repression of gene transcription.[27–31] Thus, the aim of the present study was to investigate the relative importance of these two different types of G-quadruplex interactions in produc-



TMPyP4



TMPyP2



Telomestatin

FIGURE 1 Chemical structures of TMPyP4, TMPyP2, and telomestatin.

ing the overall biological activity by comparing their short-term effects using cytotoxic concentrations or their long-term effects using noncytotoxic concentrations in human pancreatic MiaPaCa cells.

MATERIALS AND METHODS

Drugs and Cell Line

Stock solutions of telomestatin (1 mM), TMPyP4 (25 mM), and TMPyP2 (25 mM) were dissolved in DMSO and diluted to working concentrations with distilled water immediately before use. The pancreatic cell line MiaPaCa was purchased from American Type Tissue Culture Collection and cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 100 μg/mL of penicillin and streptomycin.

Cytotoxicity Assay

The cytotoxicity of G-quadruplex-interactive agents against MiaPaCa cells was determined by MTT assay. In brief, exponentially growing cells were seeded at a density of 3000 cells/well in 96-well plates, grown overnight and exposed to the testing compounds at different concentrations. After 96 h of exposure, the cell cultures were incubated with 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (1 mg/mL in Dulbecco's phosphate buffered saline) for 4 h at 37°C. The resulting formazan precipitate was solubilized with 200 μL of 0.04 M HCl in isopropyl alcohol. Optical density (OD) was read at 570 nm using a microplate reader (VERSAmax, Molecular Device, USA). For determination of the IC_{50} and IC_{10} values (concentrations causing 50% or 10% growth inhibition), the absorbance readings at 570 nm were fitted to the four-parameter logistic equation.

FACS Analysis of Cell Cycle

MiaPaCa cells were fixed by 0.5% paraformaldehyde in PBS and were gently resuspended in 1 mL hypotonic propidium iodide (PI) solution (50 μg/mL PI in a hypotonic sodium citrate solution with 0.3% NP-40 and 1.0 mg/mL RNase-A) at 1.0×10^6 cells/mL. Prior to flow cytometric measurements, samples were filtered through a 37 μM nylon mesh into 12 $\times$ 75 mm tubes and stored at 4°C until analysis within 24 h. All samples were analyzed with an EPICS ELITE flow cytometer (Coulter Cytometry, Miami, Florida) using a 15 MW argon ion laser operated at 6 amps of power at 488 nm. Photomultiplier tube voltage was adjusted for each control sample to position the G0/G1 peak to channel 240 on a 1024-channel presentation.

Histograms were analyzed for cell cycle compartments using MultiCycle-PLUS Version 4.0.

Anaphase Bridge Detection

1.0×10^5 pancreatic tumor cells in 2 mL of complete medium were seeded into a six-well plate containing a piece of slide in each well. Cells were allowed to adhere to slides overnight and then treated with corresponding IC_{50} concentrations of testing compounds. After 48 h exposure, medium was removed and the slides were washed once with PBS. Cells were fixed with 70% cold ethanol overnight. The fixed cells were stained with 5 μg/mL PI for 30 min, and the morphology of the nucleus was observed under a fluorescence microscope ($\times$400).

Long-term Treatment

Cells were grown in T175 tissue culture flasks at 2×10^6/flask and exposed to a noncytotoxic concentration of testing agents (2.5 μM for TMPyP4 and TMPyP2, 0.05 μM for telomestatin) or an equivalent volume of water (drug vehicle control) every 3 to 4 days. The cells in control and drug-treated flasks were trypsinized and counted using a haematocytometer, and 2×10^6 cells were reseeded for each group. Remaining cells were collected and kept at -80°C for the experiments described below.

Senescence-associated β-Galactosidase Staining

Cells (1×10^5) were plated out at indicated times from the above-described long-term exposure experiment into each well of a six-well plate containing a piece of slide and left overnight. Cells were then stained for β-galactosidase expression as a biomarker of cell senescence.[26] In brief, medium was removed and cells were washed in PBS and fixed in 1% formaldehyde/0.2% glutaraldehyde for 5 min at room temperature. After two washes in PBS, cells were incubated for 12–16 h with β-galactosidase stain solution containing 0.4 mg/mL X-gal, 4 mM potassium ferrocyanide, 4 mM potassium ferricyanide, and 2 mM $MgCl_2$ in PBS. Positive-stained cells and the total number of cells per well were counted. Positive cells were expressed as a percentage of total number per slide.

Measurements of Telomere Length

Telomere length was detected using the TeloTAGGG telomeric length assay kit (Roche, Nutley, New Jersey) according to the manufacturer's protocol. Genomic DNA was prepared from each sample. For each sample,

about 10 μg of genomic DNA was digested with RsaI/HinfI and then separated on a 0.8% (w/v) agarose gel. Following electrophoresis, the gel was denatured, neutralized, and transferred to a nylon membrane (Hybond-N+; Amersham, Arlington Heights, Illinois).[26] The transferred DNA was fixed by UV crosslinking. The membrane was hybridized with a telomere-specific digoxigenin (DIG)-labeled probe, which was incubated with anti-DIG-alkaline phosphatase and detected by chemiluminescence.

RESULTS

Effect of G-Quadruplex-interactive Agents on the Proliferation of MiaPaCa Cells

In previous studies, we have shown that telomestatin and TMPyP4 interact preferentially with intramolecular G-quadruplexes and intermolecular G-quadruplex structures, respectively, whereas TMPyP2 is a poor binder for both structures.[27,30] In initial studies, we assessed the effects of various concentrations of TMPyP4, telomestatin, and TMPyP2 on the growth of MiaPaCa cells. The cytotoxicity of these agents was determined by MTT assay after incubation of cells with increasing concentrations of drug for 96 h. As shown Figure 2, treatment with these agents inhibited MiaPaCa cell growth in a dose-dependent manner. We found that telomestatin is about 100-fold more potent in growth inhibition of MiaPaCa cells in comparison to TMPyP4 and TMPyP2. The IC_{50} was estimated to be 0.5 μM for telomestatin and $\sim$50 μM for both TMPyP4 and TMPyP2 (see Figure 2). Although the IC_{50} of TMPyP2 was estimated to be similar to that of TMPyP4, we ob-

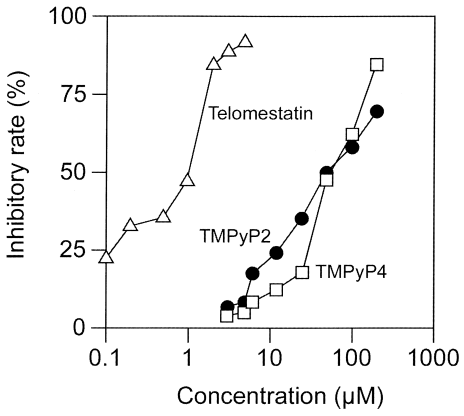


FIGURE 2 Cytotoxicity of TMPyP4, TMPyP2, and telomestatin against MiaPaCa cells. Cells were exposed for 4 days to the indicated concentrations of TMPyP4, TMPyP2, and telomestatin. After exposure, the cytotoxicity was determined by MTT assay, described under Materials and Methods. The figure shows representative results from three experiments.

served that TMPyP2 was a little less cytotoxic in MiaPaCa cells in comparison to TMPyP4 within the range of doses below the IC_{50}.

Induction of Anaphase Bridges by TMPyP4, but Not by Telomestatin and TMPyP2, in MiaPaCa Cells

During the anaphase of cell division, the replicated chromosomes separate and move to opposite poles as they prepare to form two identical sister cells.[32] However, chromosomes with dysfunctional telomeres are believed to give rise to a high rate of chromatin bridges at anaphase.[27,29] Since G-quadruplex-interactive agents can potentially cause mitotic instability, we examined the frequency of the formation of anaphase bridges by TMPyP4 and telomestatin in MiaPaCa cells. For this experiment, MiaPaCa cells were treated with these agents at IC_{50} concentration for 48 h, fixed, and stained with PI. Then the chromosome-specific effects of the testing agents were observed under a fluorescence microscope. As shown in Figure 3, the mitotic chromosomes are more diffuse and segregate abnormally, and end-to-end fusions are often observed (15–20%) in MiaPaCa cells treated with 50 μM TMPyP4. In contrast, the untreated control and the samples treated with 50 μM TMPyP2 and 0.5 μM telomestatin show normal chromosomes. This result suggests that the selectivity of G-quadruplex-interactive agents for different forms of G-quadruplex structures (intramolecular versus intermolecular) is important in inducing anaphase bridge formation during chromosome separation.

Long-term Effects of TMPyP4 and Telomestatin on the Proliferation of MiaPaCa Cells at Noncytotoxic Concentrations

To further characterize the difference in biological consequences between the two different types of G-quadruplex interactions, we examined the effect of TMPyP4, telomestatin, and TMPyP2 on long-term proliferative

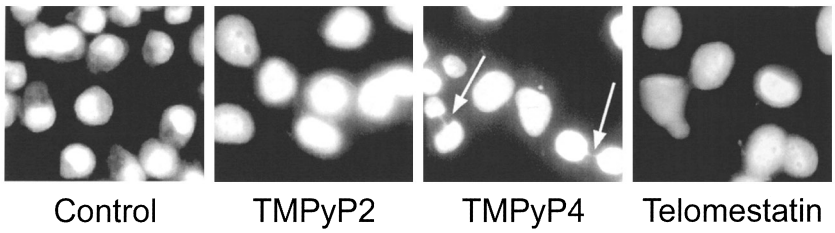


FIGURE 3 Anaphase bridges induced by TMPyP4, but not by TMPyP2 and telomestatin. Arrows indicate the anaphase bridges. After treatment with TMPyP4 (50 μM), TMPyP2 (50 μM), and telomestatin (0.5 μM) for 48 h, the PI-stained cells were observed under a fluorescence microscope ($\times$400).

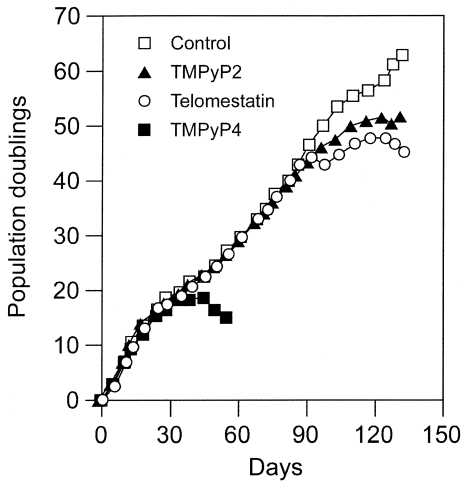


FIGURE 4 Growth arrest of MiaPaCa cells induced by long-term treatment with TMPyP4, TMPyP2, and telomestatin. Cells were treated with TMPyP4 (2.5 μM), TMPyP2 (2.5 μM), and telomestatin (0.05 μM), and the total number of cells was counted using a haematocytometer.

potential in MiaPaCa cells. For this study, MiaPaCa cells were treated for over 12 weeks with an equivalent IC_{10} dose of telomestatin (0.1 μM), TMPyP2 (2.5 μM), and TMPyP4 (2.5 μM). As shown in Figure 4, the growth arrest of MiaPaCa cells was observed within 5 weeks after treatment with TMPyP4, while cells treated with telomestatin showed the suppression of cell proliferation only after 12 weeks of treatment. The growth arrest in MiaPaCa cells by TMPyP2 was observed about 15 weeks after exposure to 2.5 μM of this compound.

Effect of TMPyP4, TMPyP2, and Telomestatin on Telomere Length in MiaPaCa Cells

Since a significant difference was observed in the long-term effects of TMPyP4, TMPyP2, and telomestatin on proliferative potential in MiaPaCa cells at IC_{10} concentrations, we next determined whether the suppression of MiaPaCa cell proliferation was accompanied by telomere erosion after long-term exposure to subtoxic concentrations of G-quadruplex-interactive agents. A southern blot analysis was done to measure telomere length in MiaPaCa cells treated with TMPyP4 and telomestatin by using the TeloTAGGG telomeric length assay kit according to the manufacturer's protocol. As shown in Figure 5, telomere length shortening to <4 Kb by TMPyP4 was observed after 39 days following exposure to a 2.5 μM concentration of drug molecules. In contrast, moderate effects on telomere shortening were produced after only 15 weeks with telomestatin treatment. TMPyP2 (2.5 μM) did not have any significant effect on telomere length,

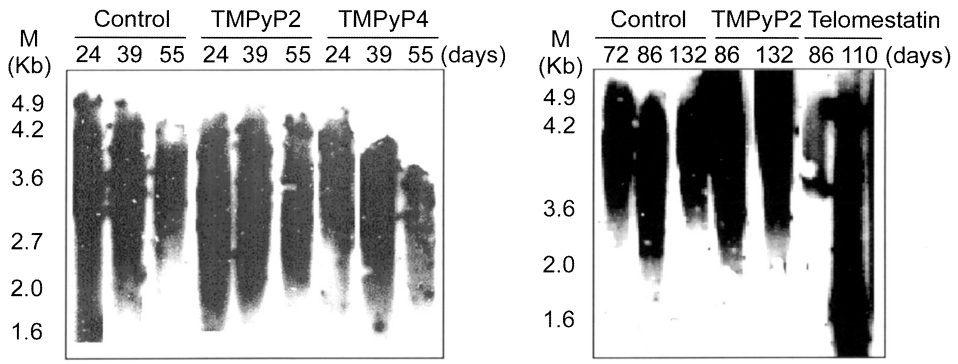


FIGURE 5 Effect of TMPyP4, TMPyP2, and telomestatin on telomere length. MiaPaCa cells were treated with TMPyP4 (2.5 μM), TMPyP2 (2.5 μM), and telomestatin (0.05 μM) and harvested at different times (days) of the culture. A southern blot analysis was used to measure telomere length by using the TeloTAGGG telomeric length assay kit according to the manufacturer's protocol.

even up to 18 weeks, suggesting that the mechanism of growth arrest by low concentrations of TMPyP2 is not likely attributable to telomerase inhibition. These results are consistent with the previous observation that the growth arrest by either telomerase inhibitors or G-quadruplex-interactive agents is associated with progressive telomere shortening.[3,4] Rather than a direct effect mediated through interaction with telomeric G-quadruplex, it is likely that the suppression of c-MYC expression by TMPyP4, which then results in inhibition of hTERT expression, may be an important component of this effect by TMPyP4.[31]

Induction of Cellular Senescence and Apoptosis by Long-term Treatment with TMPyP4 and Telomestatin at Noncytotoxic Concentrations

We also determined whether the growth arrest by subcytotoxic doses of TMPyP4 and telomestatin in MiaPaCa cells was associated with an increase in the population of senescent-like cells or apoptotic cells among treated cells. The treated cells were stained for β-galactosidase expression as a biomarker of the senescent-like cell phenotype. As shown in Figure 6A, increased expression of senescent β-galactosidase activity in MiaPaCa cells was observed coincident with growth arrest of the cells after long-term treatment with both TMPyP4 and telomestatin. The number of senescent-like cells was increased by 15% and 10% in TMPyP4- and telomestatin-treated cells, respectively, while control and TMPyP2-treated cells remained unstained. The flow cytometric analysis also revealed that 2.5 μM TMPyP4 can induce apoptosis of MiaPaCa cells after 39 days of exposure, whereas no significant apoptosis cells are detected in telomestatin- or TMPyP2-treated

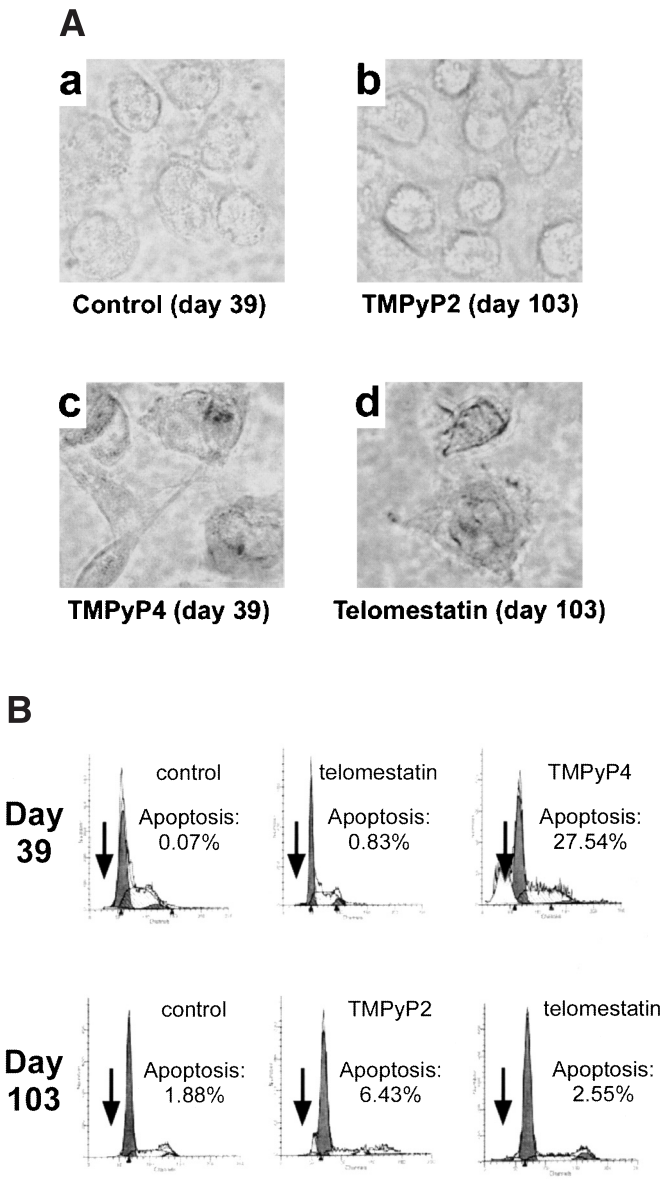


FIGURE 6 (A) Senescent-like phenotype induced by long-term treatment with TMPyP4 and telomestatin. Expression of β-galactosidase activity as a biomarker of cell senescence was measured in MiaPaCa cells harvested at day 103 (a) and in the cells treated with 2.5 μM TMPyP2 for 103 days (b), 2.5 μM TMPyP4 for 39 days (c), and 0.05 μM telomestatin for 103 days (d). Treatment with TMPyP4 and telomestatin increased the number of senescent-like cells. (B) Apoptosis induction in MiaPaCa cells after long-term treatment with TMPyP4, TMPyP2, and telomestatin. At days 39 and 103, 1×10^6 cells were fixed and stained with PI, and DNA content was measured by flow cytometry.

cells even after 103 days (Figure 6B). Overall, these results indicate that the growth arrest by TMPyP4 and telomestatin in MiaPaCa cells could be due to both telomere erosion and senescence, while the growth arrest by TMPyP2 is not associated with either senescence or telomere erosion.

DISCUSSION

In this study, our aim was to determine whether the two different G-quadruplex-interactive agents TMPyP4 and telomestatin, which bind to two different forms of the human telomeric G-quadruplex, induce different biological effects in cancer cells. Our previous studies demonstrated that telomestatin induces and stabilizes intramolecular G-quadruplex structures and prevents them from being disassembled, whereas TMPyP4 preferentially facilitates the formation of and then interacts with intermolecular G-quadruplex structures.[27,30] TMPyP4 also represses the expression of c-MYC and hTERT.[31] We therefore compared their short-term and long-term biological effects on MiaPaCa cells using cytotoxic and relatively subtoxic concentrations, respectively.

The short-term effects of TMPyP4 and telomestatin at cytotoxic concentrations are not related to progressive telomere shortening and cell senescence. For TMPyP4, these short-term effects are likely to be associated with telomere disruption and associated uncapping effects caused by the production of anaphase bridges. However, TMPyP4 is rather nonspecific in its interactions with DNA, and some of the short-term effects could simply be related to the photocleavage or cytotoxic mechanisms it shares with its positional isomer TMPyP2, which interacts poorly with G-quadruplex structures.[30] An additional mechanism could be related to interaction with the G-quadruplex in the silencer element of c-MYC which leads to downregulation of c-MYC and hTERT,[31] possibly adding to the telomere disruption mechanism produced by anaphase bridges. In previous studies,[33] the decreased cellular proliferation was observed in MCF-7 cells after repression of c-MYC expression in MCF-7 cells using c-MYC antisense oligonucleotides, supporting our hypothesis that the interaction with G-quadruplex in the c-MYC promoter also could be involved in the short-term cytotoxic effects of TMPyP4. These multiple mechanisms could be responsible for the short-term cytotoxic effects of TMPyP4 that we observe here and for the in vivo activity of TMPyP4 in xenograft model systems.[34]

Telomestatin is far more specific for G-quadruplex DNA over duplex and single-stranded DNA than TMPyP4,[35] it is unusual in that it not only binds to the intramolecular basket form of the human telomeric sequence but also facilitates its formation.[36] Although telomestatin binds to the G-quadruplex in the c-MYC promoter, it has not been shown to downregulate c-MYC (unpublished results). Nevertheless, telomestatin does

bind to a wide range of G-quadruplex, although usually less avidly than to the human telomeric G-quadruplex structure.[35] At cytotoxic concentrations, the biological effects of telomestatin could be due to interaction with both telomeres and other, yet undefined G-quadruplex structures in other biologically relevant genomic regions.[37]

The interaction of telomestatin and TMPyP4 with intramolecular versus intermolecular G-quadruplexes led to important differences in the biological effects of these drugs. Thus, TMPyP4, but not telomestatin induces a high rate of chromatin bridges at anaphase at equivalent IC_{50} concentrations. These anaphases bridge could potentially lead to genomic instability, chromosome loss, and chromosome rearrangement through chromatin fragmentation or kinetochore-spindle detachment.[32] Previously, we have also demonstrated that TMPyP4 induces anaphase bridges in sea urchin embryos, whereas telomestatin did not have this effect, which is consistent with our current observation made using MiaPaCa human pancreatic cancer cell line.[27,29] The results from both studies led us to conclude that the selectivity of telomestatin for intramolecular G-quadruplex structures and TMPyP4 for intermolecular G-quadruplex structures is important in mediating different biological effects, such as the formation of anaphase bridges.

Our results indicate that long-term treatment with both TMPyP4 and telomestatin at subtoxic concentrations results in growth arrest in MiaPaCa cells accompanying cellular senescence and telomere erosion. Growth arrest in MiaPaCa cells was also observed about 15 weeks after long-term treatment with TMPyP2. However, the mechanism of growth arrest by low concentrations of TMPyP2 is not likely attributable to telomerase inhibition for the following reasons. First, telomere erosion by TMPyP2 did not occur while there was a significant decrease in telomere length by both TMPyP4 and telomestatin in MiaPaCa cells (see Figures 5 and 6). Second, there was no increase in population of senescent-like cells or apoptotic cells in TMPyP2-treated cells. This study also revealed that there is a significant difference between TMPyP4 and telomestatin in long-term effects on MiaPaCa cells at subtoxic concentrations. The growth arrest of MiaPaCa cells by TMPyP4 was observed much earlier than that by telomestatin at subtoxic concentrations. In addition, a significant amount of apoptosis cells was detected in MiaPaCa cells 39 days after exposure to TMPyP4, whereas no significant apoptosis cells are detected in telomestatin-treated cells. Increased apoptosis could be associated with abrupt telomere erosion in MiaPaCa cells treated with TMPyP4, since telomere shortening by telomerase inhibition has been reported to induce apoptosis.[38,39] Telomerase inhibition by telomestatin is attributable to its ability to interact directly with G-quadruplex structures and thereby sequester single-stranded $d(TTAGGG)_n$ primer molecules required for telomerase activity.[36] This then leads to progressive telomere erosion and subsequent induction of cellular senescence and cell death.

Telomeres consist of tandem arrays of telomeric TTAGGG repeats complexed with specific DNA binding proteins.[40] Among them, TRF1 and TRF2 are known to bind to the double-stranded telomeric TTAGGG repeats,[41] while the single-stranded telomeric DNA at the 3′-end is specifically bound by hPot1.[42] Alternatively, TMPyP4 might be more efficient than telomestatin in disrupting the interaction of double- or single-stranded telomeric DNA with these telomeric DNA binding proteins. Indeed, previous studies demonstrated that disruption of the normal function of TRF2 or hPot1 using a dominant negative mutant or siRNA, respectively, could lead to telomere dysfunction and the formation anaphase bridges in mitosis, which are observed in TMPyP4-treated MiaPaCa cells.[43,44] In future studies, we plan to investigate the effects of different G-quadruplex ligands on the interaction of TRF2 and hPot1 with telomeric DNA to clarify this issue.

In conclusion, our data demonstrate that the selectivity of telomestatin for intramolecular G-quadruplex structures and TMPyP4 for intermolecular G-quadruplex structures is important in mediating different biological effects, such as the formation of anaphase bridges, growth arrest, senescence, apoptosis, and telomere length shortening in cultured human tumor cells. The results from this study further validate the current notion that the growth arrest of cells after prolonged exposure to telomerase inhibitors or to noncytotoxic concentrations of G-quadruplex-interactive agents is primarily caused by the progressive loss of telomere DNA and subsequent induction of cellular senescence and cell death. We propose that G-quadruplex-interactive compounds, which are designed to interact preferentially with intermolecular G-quadruplex structures of telomeric DNA, could be more effective in disrupting telomere maintenance mechanisms in human tumor cells than those that facilitate the formation of intramolecular G-quadruplex structures and subsequently inhibit telomerase activity. Last, the results from this study support our previous suggestion that highly specific and potent G-quadruplex-interactive agents could be promising agents for cancer chemotherapy.

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