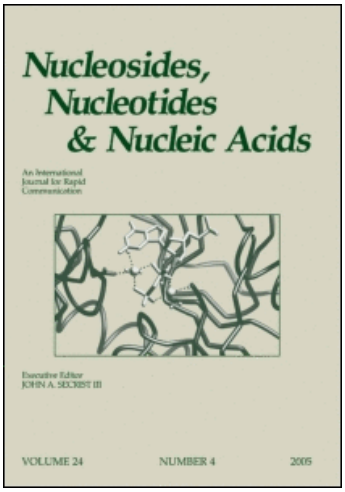

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TELOMERE SHORTENING IN HUMAN HL60 CELLS BY TREATMENT WITH 3′-AZIDO-2′,3′-DIDEOXYNUCLEOSIDES AND TELOMERASE INHIBITION BY THEIR 5′-TRIPHOSPHATES

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□ *Telomerase is thought to play an important role in the mechanism of tumor cell immortalization by maintenance of telomere length. To obtain information on the susceptibility of telomerase to nucleoside analogues, the effects of base-modified 3′-azido-2′,3′-dideoxynucleoside triphosphates on the enzyme were investigated. It is suggested that the 2-amino group of the nucleotide purine nucleus is important for the inhibitory activity. Telomere shortening caused by long-term treatment with these nucleosides is also described.*

Keywords Telomerase; reverse transcriptase; telomere shortening; nucleoside analogues; 2-amino-3′-azido-2′,3′-dideoxyadenosine

INTRODUCTION

Telomeres, the ends of eukaryotic chromosomes, contain linear chromosomal DNA consisting of G-rich repeats, TTAGGG, in vertebrate cells. In somatic cells, DNA replication at chromosome ends during successive rounds of cell division causes progressive telomere shortening and subsequent cellular senescence.[1,2] Telomerase is thought to counteract this progressive shortening and play an important role in the mechanism of tumor cell immortalization by maintenance of telomere length.[1,3,4] In order to obtain selective inhibitors that can be employed as useful tools for studying telomerase, we have been investigating the susceptibility of the

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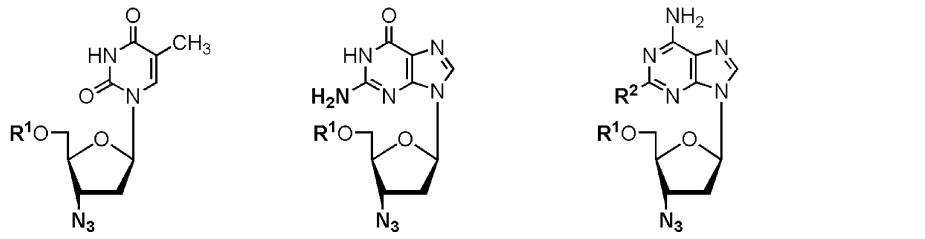

AZT: R^1=H
AZTTP: R^1=triphosphate

AZddG: R^1=H
AzddGTP: R^1=triphosphate

AZddAA: R^1=H, R^2=NH_2
AZddAATP: R^1=triphosphate, R^2=NH_2
AZddA: R^1=H, R^2=H
AZddATP: R^1=triphosphate, R^2=H

SCHEME 1

enzyme to nucleotide analogues. Telomerase is a cellular endogenous reverse transcriptase (RT) that uses its internal RNA as a template for the DNA extension reaction.[1] The triphosphate of 3′-azido-2′,3′-dideoxythymidine (AZTTP), an HIV-1 RT inhibitor, is already known to be a telomerase inhibitor.[5] We have demonstrated that a guanine counterpart of AZTTP, 3′-azido-2′,3′-dideoxyguanosine 5′-triphosphate (AZddGTP), shows potent inhibitory activity against human telomerase.[6] The present article describes the inhibition of telomerase by the adenine counterparts of these nucleotide analogues (Scheme 1), and their induction of telomere shortening in cultured cells.

MATERIALS AND METHODS

Nucleoside analogues. AZTTP and 3′-azido-2′,3′-dideoxyadenosine 5′-triphosphate (AZddATP) were purchased from TriLink BioTechnologies, San Diego, CA. AZddG and 3′-azido-2,6-diaminopurine-2′,3′-dideoxyadenosine (AZddAA) were synthesized by transglycosylation of protected AZT with N^2-acetylguanine or 2-amino-6-chloropurine, followed by ammonolysis.[7,8] Each nucleoside was chemically converted to the corresponding 5′-triphosphate, AZddGTP and AZddAATP, respectively.

Assay of telomerase, cell culture and detection of telomere. Assay of telomerase activity was carried out according to the method described previously.[9] HL60 cells (obtained from RIKEN BioResource Center, Tsukuba, Japan) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C under 5% CO_2. Cultures were grown in 25-cm^2 flasks, containing 6 ml medium per flask, without or with nucleoside analogues in the presence of 0.005% dimethylsulfoxide. Cells were transferred every four days at 0.3×10^5 cells/ml for seeding into fresh control medium or medium

containing analogue. Analysis of telomere length was performed by Southern hybridization analysis as described previously.[10]

RESULTS AND DISCUSSION

We investigated the inhibitory effects of AZddAATP and AZddATP on human telomerase in comparison with that of AZddGTP (Figure 1a). Telomerase activity was measured in the presence of inhibitors. The relative amounts of DNA products were estimated by the stretch PCR method.[9] The IC_{50} value of AZddAATP (8 μM) for human telomerase was five times lower than that of AZddATP (40 μM) (Figure 1a), suggesting that the 2-amino group of AZddAATP is effective for the inhibitory activity. As shown in Figure 1b, increasing dATP concentration weakened the inhibitory effect of AZddAATP, suggesting that AZddAATP is a competitor of dATP, not dGTP. The inhibitory effect of AZddAATP was a little weaker than that of its guanine counterpart (AZddGTP).

AZT, AZddG, and AZddAA did not show cytotoxicity for growth of human HL60 cells at a concentration of 50 μM (data not shown). We then investigated whether treatment of HL60 cells with these compounds caused a change in telomere length. Prolonged treatment of HL60 cells with 25 μM AZddAA caused telomere shortening comparable to that induced by AZddG (Figure 2a and 2b). On the other hand, AZT showed weaker

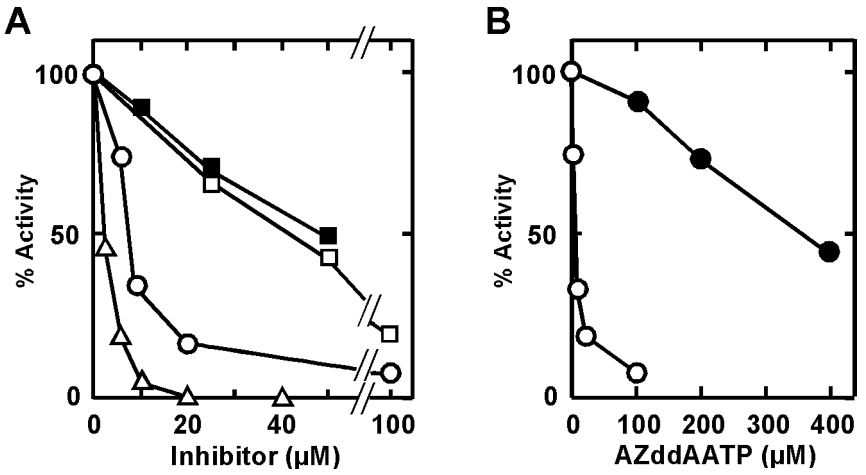

FIGURE 1 Inhibition of telomerase activity by AZTTP, AZddGTP, AZddAATP, and AZddATP in the presence of dATP, dGTP and dTTP as substrates. (a) Remaining activities in the presence of 10 μM dTTP for AZTTP (solid squares), 10 μM dGTP for AZddGTP (clear triangles) or 10 μM dATP for AAddAATP (clear circles) and AAddATP (clear squares) and the other two dNTPs each at 200 μM are shown. (b) Inhibition of telomerase activity by AZddAATP. Remaining activities in the presence of 10 μM dATP, 200 μM dTTP, and 200 μM dGTP (clear circles) and 200 μM dATP, 200 μM dTTP and 10 dGTP μM (solid circles) are shown. Activity without inhibitor was taken as 100%.

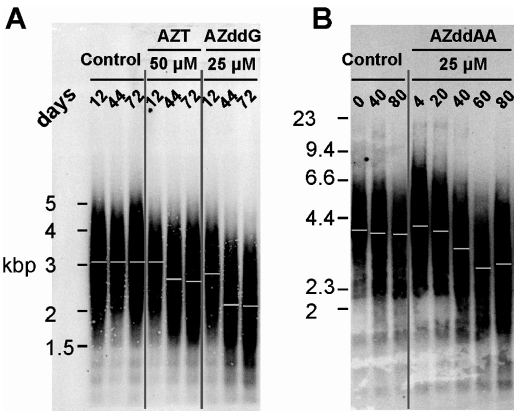

FIGURE 2 DNAs from serial passages of HL60 cells grown in the presence of 50 μM AZT, 25 μM AZddG (a) or 25 μM AZddAA (b) were digested with *Hin*f I, run on a 1% agarose gel, and Southern blotted with a digoxigenin-labeled telomere DNA probe.

activity than AZddG on telomere shortening. The telomere shortening activities of these three compounds appeared to be correlated with their telomerase-inhibitory activities.

CONCLUSION

Displacement of 2-H of AZddATP with an amino group increased its inhibitory activity on telomerase, although this modification did not affect the activity for HIV-1 RT. AZddAA treatment of HL60 cells had a more potent effect on telomere shortening than AZT treatment. Further screening of telomerase inhibitors and characterization of the inhibitor-treated cells are now underway in our laboratory.

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