Pergamon

European Journal of Cancer, Vol. 35, No. 6, pp. 1027–1034, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00037-4

Original Paper

Inhibition of Telomerase Activity and Cell Proliferation by a Reverse Transcriptase Inhibitor in Gynaecological Cancer Cell Lines

J. Murakami, N. Nagai, K. Shigemasa and K. Ohama

The Department of Obstetrics and Gynecology, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan

Telomerase is a ribonucleoprotein which has a RNA template to bind and extend telomere ends, so prolonging the life of tumour cells. The aim of this study was to determine whether transcriptase function of telomerase could be inhibited by the reverse transcriptase inhibitors (RTI); azydothymidine (AZT), dideoxyinosine (ddI) and AZT-5′ triphosphate(AZT-TP). We examined their effects on the proliferation of cancer cells and the antitumour effects of cisplatin *in vitro*. The three agents did not cause major changes in telomerase activity or telomere length in MCAS cells. However, in HEC-1 cells changes in telomerase activity and telomere length were observed that were dependent on the RTI concentration and duration of exposure. ddI and AZT-TP reduced telomerase activity and shortened the length of the telomere. In the presence of RTI, the antitumour effects of cisplatin were enhanced. This was particularly evident in HEC-1 cells where there was a marked reduction in cell proliferation, appearance of morphological changes and senescent-like cells in the presence of ddI or AZT-TP. In MCAS cells, *TP53* expression was increased by ddI and AZT-TP, while *p21* expression was unchanged. In HEC-1 cells the expression of both *TP53* and *P21* was increased by ddI. Continuous administration of RTI enhanced the cell growth inhibition of cisplatin. RTI also inhibited the proliferation of some cells. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: telomerase, telomere, RTI, enhancement of cisplatin, inhibition of cell growth
Eur J Cancer, Vol. 35, No. 6, pp. 1027–1034, 1999

INTRODUCTION

TELOMERES ARE the extreme ends of chromosomes, and their length is continuously reduced because of incomplete replication at the time of cell division. As the telomere shortens with cell divisions, the chromosome becomes unstable and cell senescence occurs.

Telomerase is a ribonucleoprotein which has a RNA template, binds and extend telomere ends. Telomerase activity is not detected in many normal adult somatic cells, but it is detected in germ cells, stem cells and cancer cells. Telomerase activity is extremely high in human cancer, and many attempts have been made to utilise telomerase activity in the diagnosis and management of cancer [1–5].

Correspondence to J. Murakami.
Received 11 Sep. 1998; revised 4 Jan. 1999; accepted 22 Jan. 1999.

Telomerase activity generally correlates with growth rate [6] and reflects differences in proliferation between tumour and normal cells [7]. Therefore, it should be possible to inhibit cell proliferation by suppressing telomerase activity. Several reports have described attempts to inhibit telomerase activity using reverse transcriptase inhibitors [8–11]. Telomerase activity can be inhibited and the telomere length shortened by retrovirus reverse transcriptase inhibitors such as azidothymidine (AZT), dideoxyguanosine (ddG), dideoxyinosine (ddI), arabinofuranyl-guanosine (Ara-G) and AZT-5′ triphosphate (AZT-TP) [8, 10]. We reviewed agents which are actually used clinically at present or agents which are going to be released soon. In the present study we used reverse transcriptase inhibitors to inhibit the transcription function of telomerase and examined the effect on the proliferation of cancer cells *in vitro*. We also examined their effect

Table 1. *Effect of RTI in MCAS and HEC*-1 *cells*

Cell line	RTI	IC_{50} (μM)	Inhibition efficiency: Concentration (μM)	Inhibition efficiency: Cell growth (MTT assay) inhibition (%)
MCAS	AZT	900	100	22.5
	ddI	400	10	18.4
	AZT-TP	65	5.0	21.2
HEC-1	AZT	1100	90	24.4
	ddI	200	10	23.1
	AZT-TP	50	4.0	19.6

in vitro on the antitumour action of cisplatin, a drug widely used in the treatment of gynaecological and other tumours. Finally, we examined whether long-term administration of reverse transcriptase inhibitors affected expression of the *TP53* and *p21* gene since these genes are thought to play a role in tumour cell growth and senescence.

MATERIALS AND METHODS

Cell lines

The human MCAS ovarian mucinous cystadenocarcinoma cells and HEC-1 uterine endometrial carcinoma G2 cells were obtained from the Japanese Cancer Research Resources Cell Bank. These cells were maintained al 37°C under 5% CO_2 in RPMI 1640 (Gibco BRL, Grand Island, New York, U.S.A.) and 10% fetal calf serum supplemented with penicillin (100 U/mL), and streptomycin (100 μg/mL) or gentamicin (50 μg/mL). Cells were counted with a haemocytometer and passaged every 4 to 6 days (five to seven population doublings) by reseeding 5×10^4 cells per flask into fresh medium.

The nucleoside analogues, azidothymidine (AZT), dideoxyinosine (ddI) and AZT-5′-triphosphate (AZT-TP), were purchased from Sigma (St Louis, Missouri, U.S.A.). Cells were harvested cells at various time points, pelleted, frozen in liquid nitrogen and stored at − 80°C.

Telomeric repeat amplification protocol assay

Preparation of cell extracts. The telomeric repeat amplification (TRAP) assay and the quantification of telomerase activity were performed as descrived previously [12]. The TRAPeze telomerase detection kit (Geron, Maryland, U.S.A.) was used according to the manufacturers instructions (Oncor, Gaithersburg, Maryland, U.S.A.) with minor modifications [13]. Cells were lysed, incubated on ice for 30 min, and then the lysate was centrifuged at 15 000×g for 20 min at 4°C. The resulting supernatant fluid was transferred to a microtube, frozen and stored at − 80°C. For the PCR reaction 1 to 2 μL of extract (5 μg of protein) was added to the 48 μL reaction mixture that contained 2 U Taq DNA polymerase. After incubation at room temperature for 30 min for the telomerase extension reaction, the samples were subjected to 31 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 45 sec. PCR products were electrophoresed, and the gel was stained with SYBR™ Green 1 (Molecular Probes Inc., Oregon, U.S.A.). Ladder formation was observed under a 254 nm transilluminator.

TRAP assay quantification was done as previously described [13]. The intensity of bands with 6-base periodicity above the internal control band as assessed by the PhosphoImaging System (EDAS system) BioMax 1D software from Kodak (Rochester, New York, U.S.A.). After subtraction of background the sum of the total pixels for all bands was divided by the pixels of the internal control band to determined a relative telomerase activity.

Cell growth inhibition by anticancer agents

On day 0 exponentially growing cells were harvested with trypsin (0.05%): EDTA (0.02%) and resuspended to a final

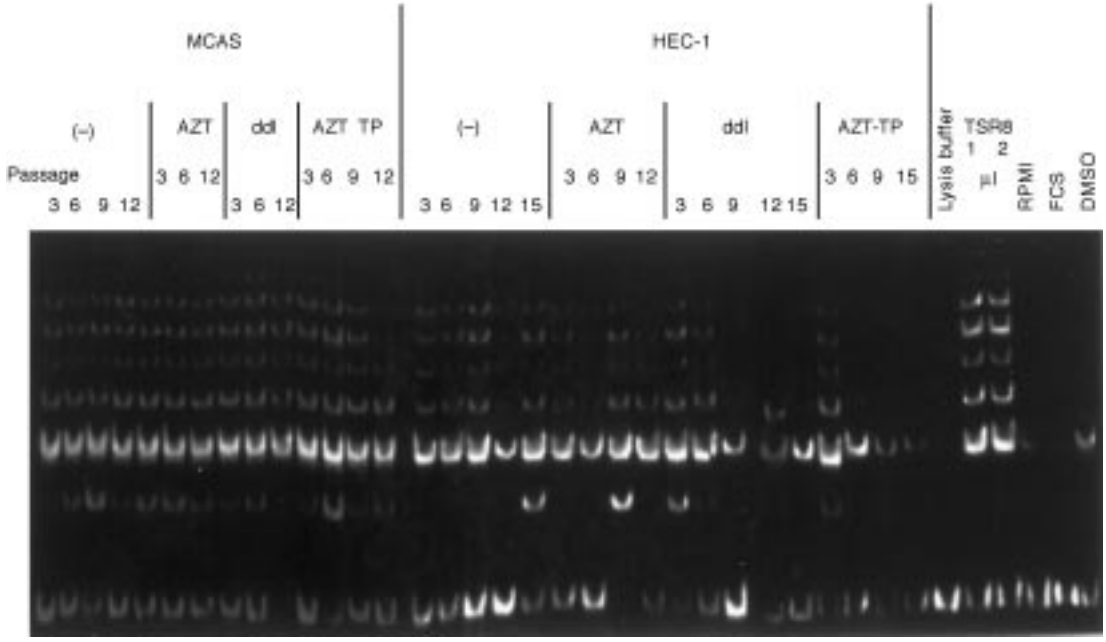

Figure 1. Inhibition of telomerase activity by RTIs. Telomerase activity was detected by the TRAP assay. TSR8 control template was used as a positive control and lysis buffer alone as a negative control. (–), medium without RTI. The band at the bottom is an internal control band of 36 bp.

concentration of 1.0×10^3 cells per mL in fresh medium with 1% FCS. Cell suspensions (100 μL) were dispensed into the individual wells of a 96-well tissue culture plate (Falcon, Franklin Lakes, New Jersey, U.S.A.). The cells were allowed to attach overnight, then cisplatin (50 μL) at different concentrations as added to individual wells. After 24-h incubation with the drug, this medium was replaced with fresh medium.

MTT assay

Viable cell growth was determined by a modified 3-(4,5-dimethylthiazol-2-yl), 5-diphenyltetrazolium bromide (MTT) assay as described previously [14]. 50 μL of MTT (1 mg per mL) was added to each well. The supernatant was removed after 4 h at 37°C. Acidified isopropanol (0.04 M HCl) (150 μL) was added and the solution was mixed thoroughly by repeated pipetting with a multichannel pipetter. The solution was maintained at room temperature for 15 min to solubilise the MTT-formazan product. Absorbance was measured with an ELISA plate reader at 570/630 nm (test/reference).

Southern blot analysis

Genomic DNA was prepared from normal and tumour tissue with a DNA Extraction Kit (Strategene, La Jolla, California, U.S.A.) and digested overnight with Hin I at 37°C. The resulting fragments (5 μg of DNA) underwent 0.7% agarose gel electrophoresis and then transferred to

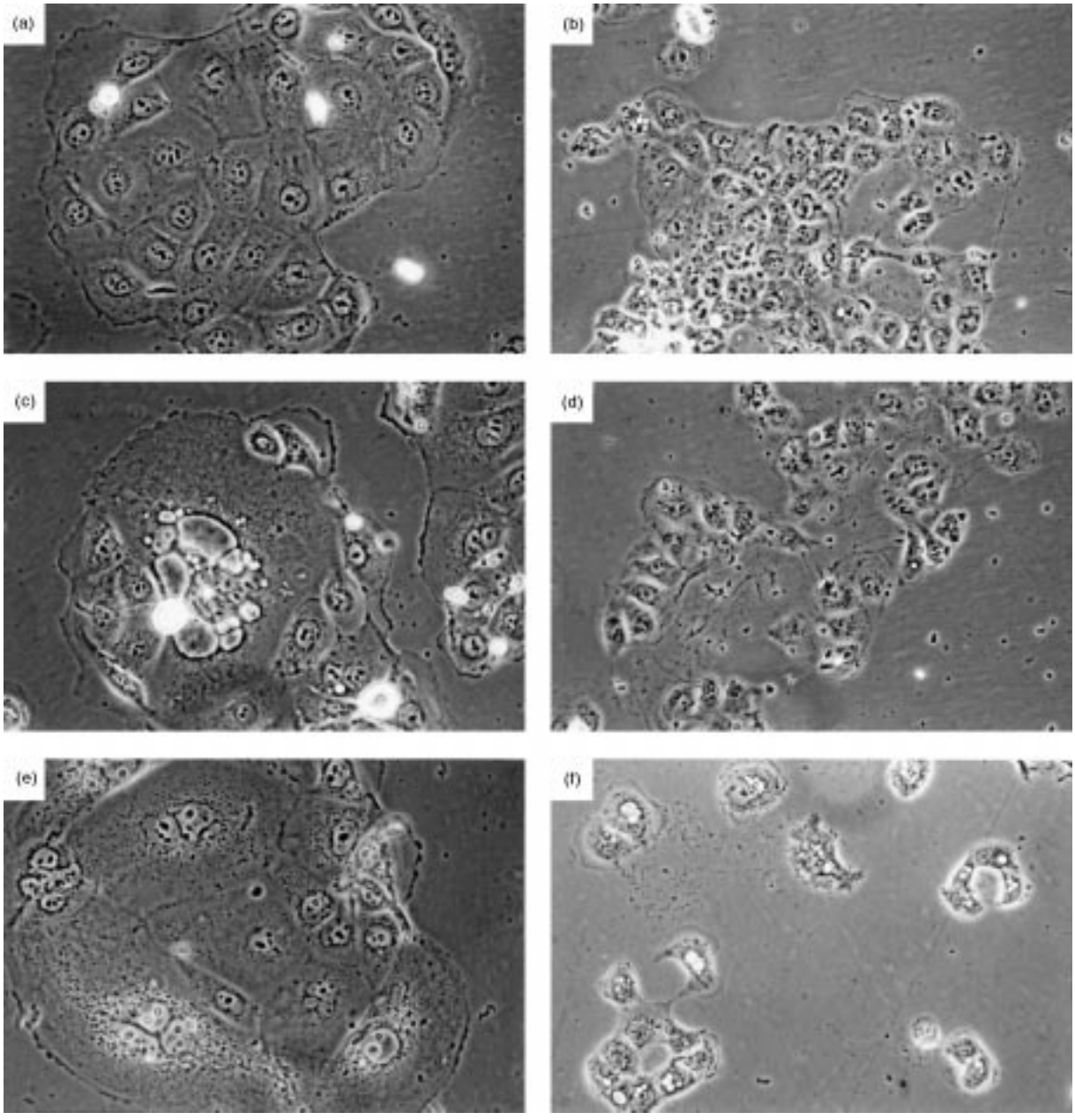

Figure 2. Changes in cell morphology in the presence of RTI. The cellular morphology of the HEC-1 cell line (a,c,e), and MCAS cell line (b,d,f) at the 12th passage in the presence of AZT (a,b), ddI (c,d), AZT-TP (e,f). HEC-1 cells displayed some morphological changes consistent with cellular senescence, including cell enlargement, flattening, containing definite vacuoles (c), and multinucleated cells appeared (e). In MCAS cells, the cytoplasm was irregular (b,d,f) and cytoplastic vacuoles were shown (d,f). Enlarged cells were not observed. a,c,e: × 400 magnifications. b,d,f: ×200 magnifications.

nylon membranes (Hybond N: Amersham, Little Chalfont, Buckinghamshire, U.K.). The membranes were incubated overnight at 37°C with α-^{32}p end labelled $(CCCTAA)_3$ telomeric probe [15], were washed twice at 37°C with 2× standard saline citrate (0.1% SDS), and then subjected to autoradiography at −80°C for 12 h. The mean length of the terminal restriction fragments (TRFs) was estimated from the position of the maximal signal [16].

RNA analysis by RT-PCR

mRNA was prepared with a Quick Prep micro mRNA purification kit (Phalmacia Biotech, Uppsala, Sweden), and the mRNA was quantified by UV spectrophotometry. cDNA was synthesised from 0.5 μg of mRNA in a 10μL reaction mixture containing MMLV reverse transcriptase buffer, 0.2 mM dNTPs, 20 pmol random hexamer primers, 10 U RNase inhibitor and 200 U MMLV reverse transcriptase (Toyobo, Osaka, Japan). After incubation at 37°C for 60 min, the reaction was heated to 94°C for 2 min, and then reaction volume was brought to 50 μL. A cDNA amount representing 50 ng of mRNA was subjected to PCR in a final volume of 25 μL that included 1 U of Taq polymerase (Toyobo) and 10 pmol oligonucleotide primers for target and control genes. The following oligonucleotide primers were used [17].

Target:
TP53
sense 5-AGGCGCTGCCCCACCA-3
antisense 5-TTCCGTCCCAGTAGATT-3
p21
sense 5-GCCGAAGTCAGTTCCTT-3
antisense 5-TCATGCTGGTCTGCCGC-3
control:
β-tubulin
sense 5-TGCATTGACAACGAGGC-3
antisense 5-CTGTCTTGACATTGTTG-3

PCR conditions were denaturation at 95°C for 30 sec, annealing at 62°C for 1 min and extension at 72°C for 1 min. PCR products were separated by 12% polyacrylamide gel electrophoresis, and bands were visualised by ethidium bromide staining and UV transillumination. The target gene expression levels were expressed as a ratio of the target to control band by a phosphorimaging system. RT-PCR analysis of each sample was done three times to arrive at a mean value for the target/control ratio.

RESULTS

RTI inhibition of proliferation

RTI (AZT, ddI and AZT-TP) was added at various concentrations to MCAS and HEC-1 cells. Cell proliferation after 72 h of incubation was assessed by the MTT assay (Table 1). Both the cell lines demonstrated reduced proliferation with increasing concentrations of RTI. Inhibition of approximately 20% was observed. Concentrations of AZT (100 μM), ddI (10 μM) and AZT-TP (5.0 μM) were selected for subsequent experiments.

Inhibition of telomerase activity and morphological change

AZT and ddI did not alter telomerase activity in MCAS cells, and the activity did not decrease with increased passage number. However, AZT-TP caused a slight decrease in telomerase activity at the 12th passage (Figure 1). In HEC-1 cells, ddI and AZT-TP caused a decrease in telomerase activity in relation to passage number. The time between passages increased, so while cells were passages every 3 to 4 days initially, this was extended to every 6 to 7 days by the 10th passage. Enlarged cells and cells with definite vacuoles increased and the rate of cell proliferation decreased sharply along the repetition of passaging especially in the presence of ddI and AZT-TP (Figure 2).

Relative telomerase activity

Almost no change in activity was seen when MCAS cells were passaged in the absence of RTI. The maximum decrease in activity was 12.6% for AZT, 8.3% for ddI and 32.5% for AZT-TP (Figure 3). In HEC-1 cells the maximum decrease in activity was 32.7% for AZT, 70.3% for ddI and 91.2% for AZT-TP (Figure 3).

Change in telomere length

Changes in telomere length (TRF-terminal restriction fragments) with incubation and passage in the presence of RTI were assessed (Figure 4). In MCAS cells, TRF length showed no clear change with passage in the absence of RTI (mean TRF length: 8.3 to 6.4 kbp), and there was no major change in the presence of AZT (mean TRF length: 6.2 kbp). With ddI there was a slight shortening with cell passage, and the mean TRF length decreased by 27.3% (6.6 kbp before passage to 4.8 kbp after 12 passages). The mean TRF length in the presence of AZT-TP decreased by 34.9% (6.3 kbp before passage to 4.1 kbp after 12 passages).

In HEC-1 cells, TRF length showed no clear change with passage in the absence of RTI (mean TRF length: 5.8 to 4.3 kbp). TRF length was shortened with passage in the presence of each of the RTIs. The mean TRF length in the presence of AZT decreased by 46.6% (5.8 kbp before passage to 3.1 kbp after 12 passages). The mean TRF length in the presence of ddI decreased by 57.9% (5.7 kbp before passage to 2.4 kbp after 12 passages). In the presence of AZT-TP it decreased by 33.3% (5.7 kbp before passage to 3.8 kbp after 12 passages).

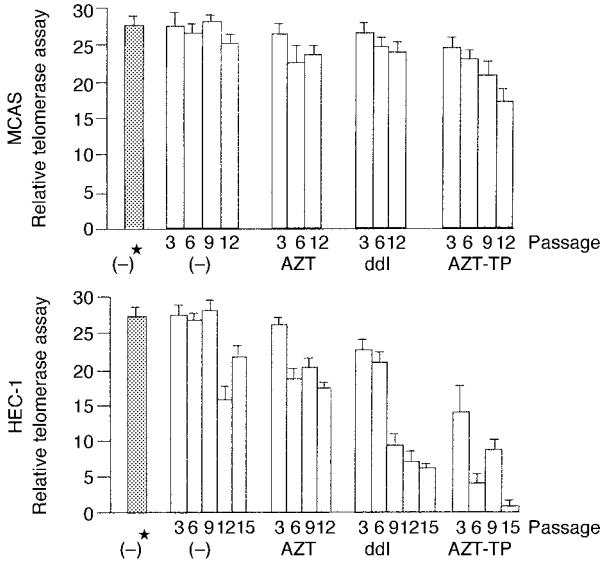

Figure 3. Effect of reverse transcriptase inhibitors on relative telomerase activity. Relative telomerase activity was calculated as the ratio between the sum total of pixel intensity of every 6 bp band and the pixel intensity of internal control band. (–) medium without RTI. *A sample of the cells before passage.

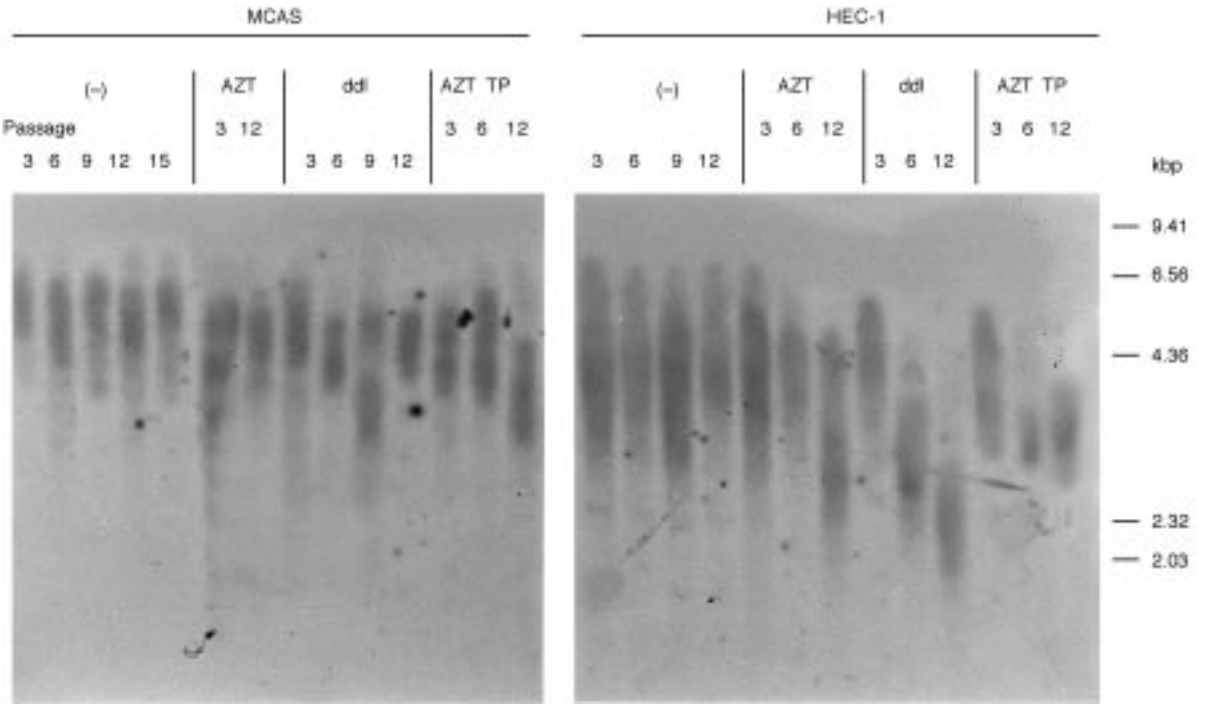

Figure 4. Effect of reverse transcriptase inhibitors on telomere length. The size of terminal restriction fragments was determined by Southern blot hybridisation. Molecular weight standards are shown on the right. (–), medium without RTI.

Effects of RTIs on the action of cisplatin

Cell proliferation was assessed 24 h after the addition of serially diluted cisplatin to cells (12th passage) (Figure 5). Cell proliferation was suppressed in direct relation to the concentration of cisplatin. In MCAS cells, cell proliferation was reduced by approximately 23% at a cisplatin concentration of 1.95 μg/mL in the presence of RTIs. No major differences in effect were seen between the RTIs.

In HEC-1 cells AZT and AZT-TP had similar modest effects on cell proliferation, whilst ddI had a more significant effect. At a cisplatin concentration of 1.95 μg/mL, AZT and AZT-TP caused a 27.3% decrease in proliferation and ddI a 43.8% decrease. At a cisplatin concentration of 15.6 μg/mL, AZT and AZT-TP caused a 32.1% decrease in proliferation and ddI an 81.4% decrease ($P < 0.005$).

Assessment of the TP53 *and* p21 *genes*

Expression of the *TP53* and *p21* genes before and after 12 passages was examined (Figure 6). In MCAS cells, *TP53* expression was minimally affected by AZT, but ddI and AZT-TP caused an increase in expression ($P < 0.005$); the expression of *p21* was essentially unaffected by the RTIs. In HEC-1 cells, the expression of *TP53* increased with ddI ($P < 0.001$) but was unaffected by AZT or AZT-TP. The expression of *p21* increased with ddI ($P < 0.001$) but was not affected by the other two drugs.

DISCUSSION

Several studies have already reported on the effects of inhibitors of telomerase activity. Norton and colleagues [18] reported the specific inhibition of template portions of the human telomerase RNA component (hTR) [19] by various peptide nucleic acids (PNAs) or phosphorothioate (PS) oligomers. PNA, in particular, demonstrated excellent inhibition of telomerase activity [18, 20]. Sun and colleagues [21] reported that telomerase activity could be inhibited by the G-quadruplex-interactive compound as a target of the nucleic acid structure. There have also been studies on the template

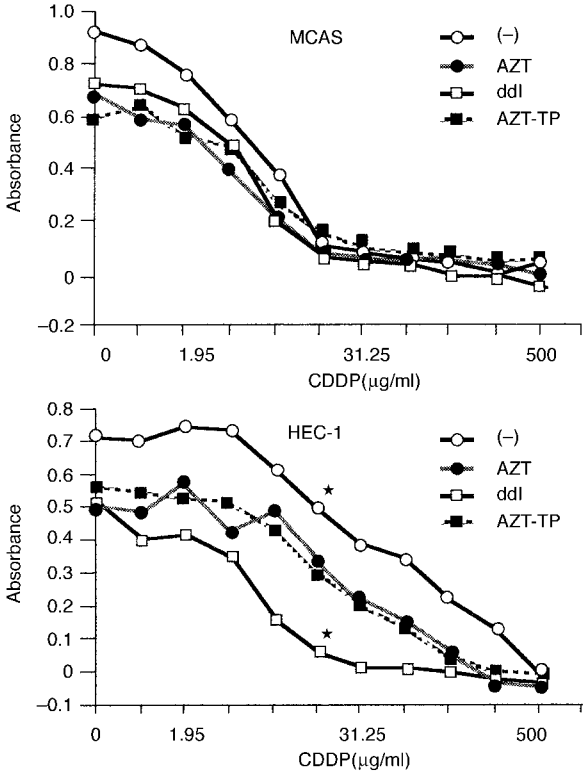

Figure 5. Inhibition of cell proliferation of cisplatin in the presence of RTI. Stepwise dilutions of cisplatin were added to cells at their 12th passage. Cell growth over 24 h was measured by MTT assay. (–), medium without RTI. *P<0.005.

portion using protein phosphate 2A [22] and the hammerhead ribozyme [23]. Strahl and colleagues demonstrated inhibition of telomerase activity in tetrahymena [8] and human T-cell and B-cell lines [10] using a nucleotide analogue which inhibits retroviral reverse transcriptase. They reported good inhibition of telomerase activity with Ara-GTP and ddGTP. Also, AZT significantly reduced telomere length and generated telomere instability. Although AZT-TP has not been shown to inhibit telomerase activity, it is thought to prevent correct binding of the enzyme which forms the telomeric sequence.

In the present study, we examined the effect on telomerase activity of long-term culture with RTI analogues. We also examined the effect of RTI analogues on the susceptibility of cancer cells to cisplatin, a widely used anticancer agent. Strahl and colleagues [8, 10] performed a telomerase activity assay using cells exposed to analogues for the short time required for primer extension and reported that inhibition of telomerase activity by ddI or AZT-TP was slight. It has also been suggested that telomerase is inhibited by AZT, because AZT is converted into AZT-TP and competes with TTP in binding to telomeres, and that ddI is also converted into ddATP intracellularly to inhibit telomerase [24]. In the present study ddI and AZT-TP significantly inhibited telomerase activity in HEC-1 cells, though they had little effect in MCAS cells. Cell proliferation decreased markedly and flatting and enlarged cells increased every time the medium containing ddI was exchanged during culture of HEC-1 cells. It is thought unlikely that these effects were achieved by selective inhibition of telomerase activity and induction of senescence, and it appears more plausible that cell proliferation was decreased by the toxicity of the analogue and telomerase activity decreased as a result. Since AZT inhibited cell proliferation irrespective of the level telomerase activity and this effect became stronger with an increase in concentration [25], AZT may also have shown an effect on telomerase activity if it was used at higher concentrations.

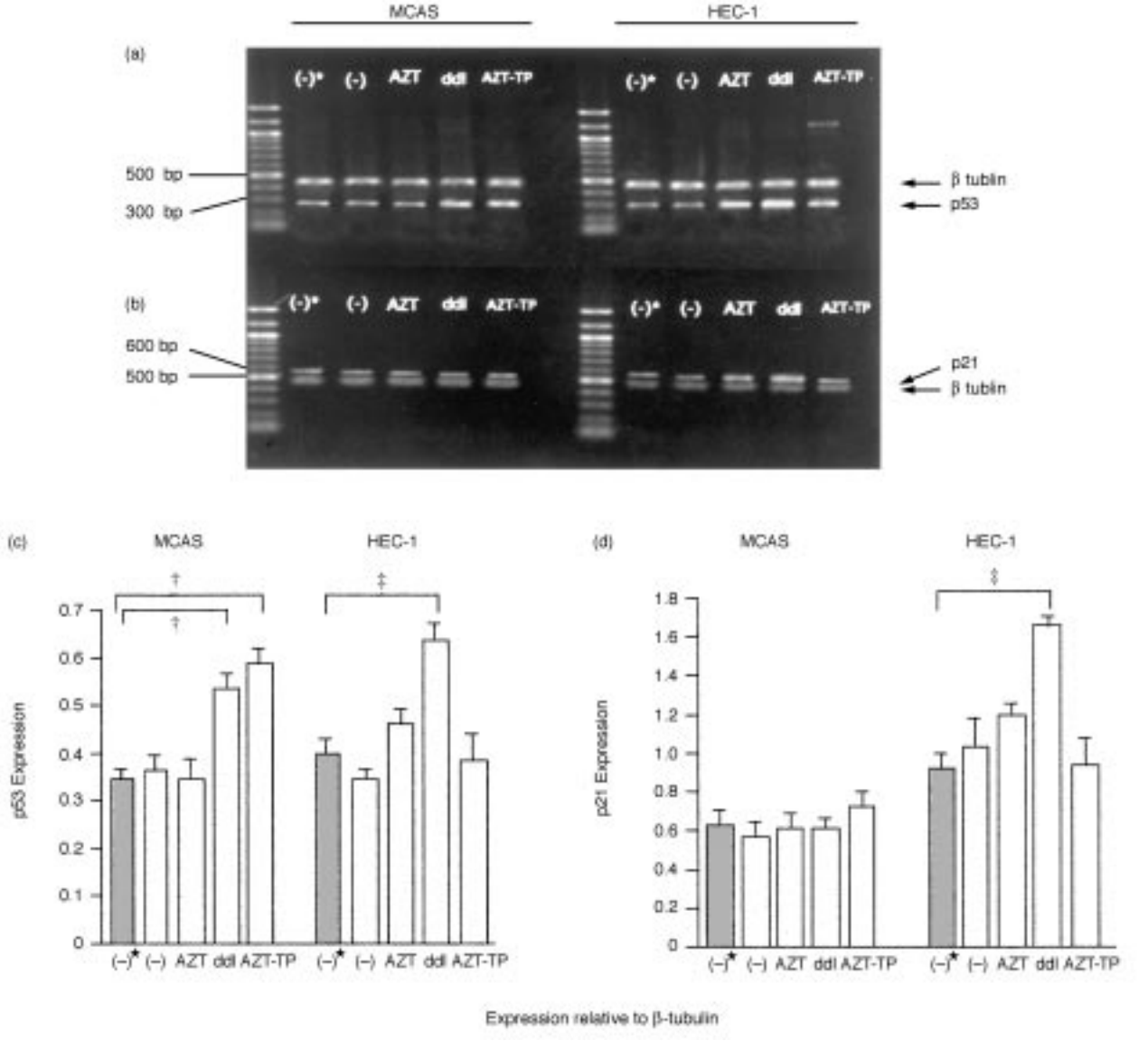

Figure 6. Expression of (a) *TP53* and (b) *p21* in MCAS of HEC1 cells. Gene expression at the 12th passage in the presence of RTI is shown. (–), medium without RTI. (c) *TP53* expression relative to β-tubulin expression. (d) *p21* expression relative to β-tubulin expression. The intensity of each band was measured densitometrically. Quantities are represented as the ratio between target and control, β-tubulin expression. *A sample of the cells before passage. †*P*<0.005, ‡ *P*<0.001.

Telomere shortening analogues such as AZT and Ara-G have caused the most rapid changes in telomere length in tetrahymena and human T and B cells, while ddI and ddG had no effects [8, 10].

AZT is thought to have an important role in the maintenance of telomeres. Many apparently senescent cells appear in long-term cultures in the presence of AZT [9], with aberrant chromosomes that may result from abnormal amplification of telomere like centrometric DNA [11]. Gomez and colleagues [26] cultured HeLa cells that were devoid of telomerase activity in the presence of AZT over a long period. According to their findings, the telomeres were shortened but there were no signs of senescence. In the present study, none of the three agents shortened telomeres in MCAS cells, but ddI and AZT-TP did markedly shorten telomeres in HEC-1 cells. This was thought to be ascribable to the decrease in telomerase activity. It is possible that telomere shortening occurred early as a result of senescent-like phenotype induction by the analogue. Further study is required to characterise the differences seen between the two cell lines.

Cisplatin is widely used in the treatment of gynaecological cancer, particularly ovarian cancer [27, 28]. However, cisplatin resistance is a problem [29, 30]. Burger and colleagues [31] targeted the telometric tandem repeat sequence, a G-rich sequence, by utilising the fact that cisplatin is associated with the G-Pt-G adduct in the formation of intrastrand lesions [32]. These investigators reported that telomerase activity was specifically reduced by inhibition of transcription of the hTR component. Therefore, we examined the effect of cisplatin administration in the presence of an RTI. We found that the antitumour effects of cisplatin were greater in both cell lines in the presence of RTIs. In MCAS cells, the antitumour effects of cisplatin did not differ between the RTIs, but in HEC-1 cells, proliferation was strongly inhibited by ddI. It is interesting that the telomerase activity of AZT-TP exposed cells were different from that of AZT in HEC-1 cells, but the antitumour effect of cisplatin was almost similar. Telomerase activity has been shown to be higher in actively dividing cells [6]. However, AZT-TP lowers telomerase activity and reduces HEC-1 cell proliferation more significantly than AZT. Therefore, the incorporation of cisplatin is reduced, and it is possible that after 24 h of exposure to cisplatin the rate of cell proliferation would be similar to that seen with AZT. The next question is why the antitumour effect of cisplatin in HEC-1 cells increases in the presence of ddI, a drug which reduces both telomerase activity and cell proliferation. Kondo and colleagues [33] transduced an antisense telomerase expression vector and not only found that telomerase activity was inhibited but also that the occurrence of apoptosis induced by cisplatin was enhanced. Since the appearance of morphological change and expression of *p21* was enhanced in HEC-1 cells exposed to ddI, it is suggested that senescene was induced. Although AZT-TP decreased telomerase activity and induced morphological changes to the same extent as ddI, an increase in *p21* expression was not observed in the AZT-TP-exposed cells. ddI may cause different changes to cells, mediated by a different mechanism to that of AZT-TP. Further study is required to understand the mechanism by which RTIs enhance the effect of cisplatin.

To intensify the effect of chemotherapy, dose is increased or multiple drugs are used. However, because of toxicities or other restrictions, it is often difficult to increase the dose. If it was possible to augment the effect of anticancer drugs by auxiliary means, chemotherapy could be more effective even at a low-dose. Since RTI reduces cell proliferation through inhibition of telomerase, it has little effect on normal somatic cells, which have no telomerase activity. Therefore, it may be that RTI could be effectively combined with anticancer drugs. Thus, the effect of ddI on HEC-1 cells observed in this study is notable. It is also necessary to investigate the effects of RTI on stem cells and germ cells, which have telomerase activity. It seems warranted to study RTI further with the intention of achieving its clinical application.

1. Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF. Telomerase activity in human breast tumours. *J Natl Cancer* 1996, **88**, 116–122.
2. Baccetti S, Counter CM. Telomeres and telomerase in human cancer (review). *Int Natl J Oncol* 1995, **7**, 423–432.
3. Breslow RA, Shay JW, Gazdar AF, Srivastava S. Telomerase and early detection of cancer: a National Cancer Institute Workshop. *J Natl Cancer Inst* 1997, **89**, 618–623.
4. Shay JW, Gazdar AF. Telomerase in the early detection of cancer. *J Clin Pathol* 1997, **50**, 106–109.
5. Murakami J, Nagai N, Ohama K. Diagnostic significance of the telomerase activity in ovarian tumours. *Cytometry Res* 1997, **7**, 39–44.
6. Holt SE, Aisner DL, Shay JW, Wright WE. Lack of cell cycle regulation of activity in human cells. *Proc Natl Acad Sci USA* 1997, **94**, 10687–10692.
7. Belair CD, Yeager TR, Lopez PM, Reznikof CA. Telomerase activity: A biomarker of cell proliferation, not malignant transformation. *Proc Nad Acad Sci USA* 1997, **94**, 13677–13682.
8. Strahl C, Blackburn EH. The effects of nucleoside analogues on telomerase and telomeres in Tetrahymena. *Nucleic Acids Research* 1994, **22**, 893–900.
9. Yegorov YE, Chernov DN, Akimov SS, Bolsheva NL, Krayevsky AA, Zelenin AV. Reverse transcriptase inhibitors suppress telomerase function and induce senescence-like processes in cultured mouse fibroblasts. *FEBS Letters* 1996, **389**, 115–118.
10. Strahl C, Blackburn EH. Effect of reverse transcriptase inhibitor on telomere length and telomerase activity in two immortalized human cell line. *Mol and Cell Biol* 1996, **16**, 53–65.
11. Parra I, Flores C, Adrian D, Windle B. AZT induces high frequency, rapid amplification of entrometric DNA. *Cytogenet Cell Genet* 1997, **76**, 128–133.
12. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994, **266**, 2011–2015.
13. Kim NW, Wu F. Advances in quantification and characterisation of telomerase activity by the telomeric repeat amplification protocol(TRAP). *Nucleic Acids Res* 1997, **25**, 2595–2597.
14. Mosmann T. Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.
15. Moyzis RX, Buckingham JM, Cram LS, *et al*. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 1988, **85**, 6622–6626.
16. Hiyama K, Ishioka S, Shirotoni Y, *et al*. Alterations in telomeric repeat length in lung cancer are associated with loss of heterozygosity in p53 and Rb. *Oncogene* 1995, **10**, 937–944.
17. Shigemasa K, Hu C, West CM, *et al*. p21: a monitor of p53 dysfunction in ovarian neoplasia. *Int J Gynecol Cancer* 1997, **7**, 296–303.
18. Norton JM, Piatyszek AP, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase activity by peptide nucleic acids. *Nature Biotechnology* 1996, **14**, 615–618.
19. Feng J, Funk WD, Wang SS, *et al*. The RNA component of human telomerase. *Science* 1995, **269**, 1236–1241.
20. Hamilton SE, Pitts AE, Katipally RR, *et al*. Identification of determinants for inhibition binding within the RNA active site of human telomerase using RNA scanning. *Biochemistry* 1997, **36**, 11873–11880.

21. Sun D, Thompson B, Cathers BE, *et al*. Inhibition of human telomerase by a G-quadruplex-interactive compound. *J Med Chem* 1997, **40**, 2113–2116.
22. Li H, Zhao L-L, Funder JW, Liu J-P. Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. *J Biol Chem* 1997, **272**, 16729–16732.
23. Kanazawa Y, Ohkawa K, Ueda K, *et al*. Hammer head ribozyme-mediated inhibition of telomerase activity in extracts of human hepatocellular carcinoma cells. *Biochem Biophy Res Comm* 1996, **225**, 570–576.
24. Pai RB, Pai B, Kukhanova M, Dutschman GE, Guo X, Cheng YC. Telomerase from human leukemia cells: properties and its interaction with deoxynucleoside analogues. *Cancer Res* 1998, **58**, 1909–1913.
25. Melana SM, Holland JF, Pogo BG. Inhibition of cell growth and telomerases activity of breast cancer cells *in vitro* by 3′-azido-3′-deoxythymidine. *Clin Cancer Res* 1998, **4**, 693–696.
26. Gomez DE, Tejera AM, Olivero OA. Irreversible telomere shortening by 3′-azido-2′,3′-dideoxythymidine (AZT) treatment. *Biochem Biophys Res Commun* 1998, **246**, 107–110.
27. Bottalico C, Lorusso V, Brandi M, *et al*. Correlation between HPLC-determined lonidamine serum levels and clinical response in patients with advanced ovarian cancer. *Anticancer Res* 1996, **16**, 3865–3869.
28. Soulie R, Bensmaine A, Garrino C, *et al*. Oxaliplatin/cisplatin (L-OHP/CDDP) combination in heavily pretreated ovarian cancer. *Eur J Cancer* 1997, **33**, 1400–1406.
29. Chollet P, Bensmaine MA, Brienza S, *et al*. Single agent activity of oxaliplatin in heavily pretreated advanced epithelial ovarian cancer. *Ann Oncol* 1996, **7**, 1065–1070.
30. Saijo N. Clinical trials of irinotecan hydrochloride (CPT, campto injection, topotecin injection) in Japan. *Ann NY Acad Sci* 1996, **13**, 292–305.
31. Burger AM, Double JA, Newell DR. Inhibition of telomerase activity by cisplatin in human testicular cancer cells. *Eur J Cancer* 1997, **33**, 638–644.
32. Bedford P, Fichtinger-Schepman AMJ, Shellard SA, Walker MC, Masters JRW, Hill BT. Differential repair of platinum-DNA adducts in human bladder and testicular tumour continuous cell lines. *Cancer Res* 1988, **48**, 3019–3024.
33. Kondo Y, Kondo S, Tanaka Y, Haqqi T, Barna BP, Cowell JK. Inhibition of telomerase increases the susceptibility of human malignant glioblastoma cells to cisplatin-induced apoptosis. *Oncogene* 1998, **16**, 2243–2248.