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European Journal of Cancer 38 (2002) 586–593

European Journal of Cancer

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Decreased telomerase activity is not a reliable indicator of chemosensitivity in testicular cancer cell lines

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Received 6 August 2001; received in revised form 4 October 2001; accepted 22 November 2001

Abstract

Telomere stabilisation is a critical step in tumorigenesis and telomerase, an enzyme which counteracts telomeric DNA loss, is active in most tumours. Conflicting evidence has been published concerning the potential use of telomerase activity as a measurement of drug-induced tumour cell killing. In this study, the time courses of telomerase loss and induction of apoptosis were investigated in two testicular cell lines, Susa CP and 833 K, following 4-h exposure to cisplatin, melphalan or doxorubicin. Telomerase activity was only affected in both cell lines at 20 h following exposure to high concentrations of cisplatin (100× the drug concentrations causing 50% growth inhibition (IC_{50} values)). The time course of melphalan-induced telomerase loss, which was again only apparent at 100× IC_{50} concentrations, varied between the cell lines and doxorubicin (100× IC_{50}) did not induce telomerase loss in either of the cell lines. Importantly, the levels and rates of appearance of apoptotic cells (nuclear morphology and annexin V staining) were similar for all three drugs in both cell lines; i.e. cisplatin, melphalan and doxorubicin (100× IC_{50}) caused similar frequencies of apoptosis in Susa CP cells at 24 h whereas telomerase activities were 65, 123 and 96% of the control, respectively. The possibility that telomerase activity was lost following cisplatin treatment through a direct interaction of cisplatin with telomerase was discounted. Additionally, the relative levels of the RNA component of telomerase (*hTR*) and mRNA for the telomerase catalytic subunit (*hTERT*) were not related to the observed decreases in telomerase activity. These data indicate that telomerase activity is not a reliable indicator of chemosensitivity in human testicular cancer cells. Furthermore, cisplatin-induced loss of telomerase activity is not due to a direct reaction with the enzyme or decreased *hTR* levels.

Keywords: Telomerase activity; Apoptosis; Chemosensitivity; Cisplatin; Melphalan; Doxorubicin; Testicular cancer

1. Introduction

The termini of linear chromosomes, telomeres, are specialised structures that are essential for chromosome integrity [1,2] and extensive loss of telomeric repeat sequences, can lead to cellular senescence [3]. Telomerase, a ribonucleoprotein reverse transcriptase, uses an internal RNA template to add telomeric repeats onto the ends of chromosomes [4,5]. Telomerase activity shows a strong association with cancer [6–8] and, although telomerase activation alone is not sufficient for malignant transformation, telomere stabilisation, primarily by telomerase, is a component of tumorigenesis [9]. The usefulness of measuring telomerase activity for diagnostic and prognostic purposes is under investigation [6,10].

Telomerase activity has also been proposed as a marker for tumour cell killing *in vitro* [11–13] and has potential as an indicator in chemosensitivity assays. A correlation between telomerase activity and chemosensitivity has been reported in human oesophageal cancer and in several human cancer cell lines [14,15]. In addition, a correlation between loss of telomerase activity and loss of cell membrane integrity in drug-treated leukaemic cells has been described [16]. However, other reports have shown a lack of correlation between drug-induced cell killing and loss of telomerase activity [17–19]. In this study, we report experiments examining loss of telomerase activity and cytotoxicity.

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0959-8049/02/$ - see front matter
PII: S0959-8049(01)00406-3

2. Material and methods

2.1. Cell lines and drug preparation

The testicular teratoma cell lines Susa CP [20] and 833K [21] were kindly provided by Professor J.W.R. Masters (University College, London, UK). Cells lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 complete medium supplemented with 10% (v/v) fetal calf serum (Gibco-BRL, Paisley, UK) at 37 °C in air containing 5% CO_2. For drug treatments, 100 mM solutions of cisplatin (Johnson Mathey, Reading, UK) and melphalan (Sigma-Aldrich Ltd, Poole, UK) were prepared in dimethylsulphoxide (DMSO) and acidified ethanol (5 M HCl:ethanol, 1:49 v/v), respectively. These solutions were immediately diluted into medium to give the required drug concentrations. The final concentrations of DMSO or acidified ethanol were $\leqslant 1\%$ (v/v). Solvents at this concentration were added to all control cultures and lysates. A 300-μM stock solution of doxorubicin (Sigma-Aldrich Ltd, UK) was prepared in water and stored at −80 °C.

2.2. Measurement and quantification of telomerase activity

Following drug exposure and any subsequent incubation, all the cells in the culture flasks were harvested by trypsinisation and washed twice with phosphate-buffered solution (PBS) by centrifugation (350g×5 min). After enzyme extraction [17], telomerase activity was measured using the telomeric repeat amplification protocol (TRAP) using 1 and 2 μg protein per assay [8,17]. Telomerase activity was also measured using the TRAP-*eze*™ kit (Oncor Inc, MD, USA) following the manufacturers' protocol using 0.3 and 1 μg protein per assay. Protein concentrations were determined using the 'Bio-Rad protein detection kit' (Bio-Rad, Munich, Germany). Radioactivity was detected using a phosphorimager™ (Molecular Dynamics Inc, Buckinghamshire, UK). Protein extracts incubated with RNase A (Gibco-BRL, 5 μg/μl) for 30 min/37 °C prior to the TRAP assay were always negative for telomerase activity.

Both telomerase assays were based on the same principle and for both, the activity was expressed as a percent of

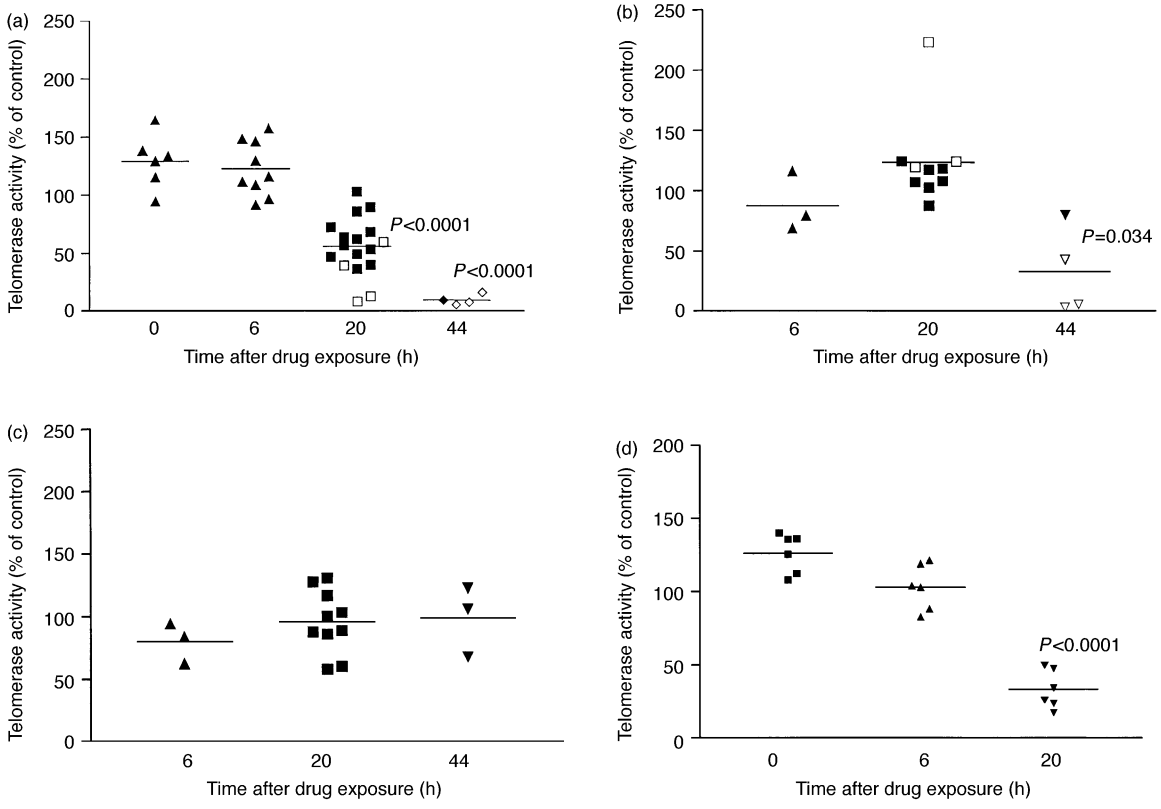

Fig. 1. Telomerase activity in Susa CP and 833K cells at various time points following a 4-h exposure to cytotoxic drugs. Each point represents the integrated intensities of telomeric repeat amplification protocol (TRAP) bands determined as percentages of the intensities of bands produced by control cell extracts. Horizontal lines represent the mean telomerase activity at each time point. P = probability that mean $<100\%$. (a) Susa CP cells treated with 100 μM cisplatin; (b) Susa CP cells treated with 100 μM melphalan; (c) Susa CP cells treated with 1.3 μM doxorubicin; (d) 833 K cells treated with 100 μM cisplatin. Closed and open symbols represent TRAP*eze* and TRAP assays, respectively.

the control activity. Equivalence of the two assays was shown by Holt and colleagues [22] and also the data in Fig. 1. Intensities of bands resulting from both TRAP assay methods were quantified using phosphorimager software (Image Quant^{TM}). The band intensities for the drug-treated cells were expressed as percentages of the intensities for control (untreated) cells for assays using the same protein concentrations. The percent inhibition of telomerase was independent of the amount of cell lysate protein added to the TRAP assay over the range used (0.01–1.0 μg).

2.3. Cell growth inhibition following drug treatment

The sensitivities of the cell lines to drug treatment were assessed by the Sulphorhodamine B (SRB) growth inhibition assay [23]. 10^4 cells were plated into each well of 96-well plates. After 24 h, the drugs were added and removed after 4 h. Cells were fixed after incubation for a further 5 days (a period over which the control cells remained in active growth). The drug concentrations causing 50% growth inhibition (IC_{50}) were calculated by fitting the Hill equation (Prism, GraphPad Software Inc., San Diego, USA) (Table 1).

2.4. Measurement of apoptotic cells

2.4.1. Using Hoechst 33258 staining

At each time point, cells suspended in medium were fixed with formaldehyde (2% (v/v) final concentration) and stained with Hoechst 33258 (5 μM). At least 300 cells per sample were scored for normal and apoptotic nuclear morphology under a fluorescence microscope.

2.4.2. Using annexin V labelling

Measurements were performed using the 'Apoptosis detection kit' (R&D Systems, Abingdon, UK) in accordance with the manufacturer's protocol.

2.5. Incubation of cell extracts with cisplatin prior to the telomerase analysis

Aliquots (10 μl) from untreated Susa CP and 833K cells extracts were incubated with cisplatin (30 min, 30×C). Following incubation, cell extracts were diluted with lysis buffer to give 0.5 μg protein/μl and 2 μl was analysed using the TRAP*eze* assay.

2.6. Measurement of platinum concentrations

Platinum was analysed using an atomic absorption spectrometer (AAS) fitted with a graphite tube atomiser (PU 9100X, ATI Unicam, Cambridge, UK) as previously described in Ref. [24]. Samples were analysed in duplicate.

2.7. Measurement of platinum bound to macromolecules in cisplatin-treated cells

Cells exposed to 100 μM cisplatin for 4 h were harvested and lysed using the TRAP*eze* extraction method. Cell extract (300 μl) was mixed with an equal volume of 10% (w/v) trichloroacetic acid (TCA) and kept on ice for 10 min. After centrifugation (8000g×5 min), the pellet was hydrolysed by incubation with 40 μl of 1 M NaOH at 60 °C overnight. The hydrolysate was acidified with HCl before the AAS analysis.

2.8. Measurement of platinum bound to macromolecules in cisplatin-treated cell extracts

Immediately following the 30-min incubation of cell extracts with cisplatin, NaCl (140 mM final concentration, to reduce the rate of further reaction) and bovine serum albumin (BSA)) (0.1 mg/ml final concentration) were added. After 10 min at 0 °C, the samples were centrifuged (8000g×5 min). The pellet was washed twice with 10% (w/v) TCA, hydrolysed with 40 μl of 1M NaOH (60 °C×18 h) and then acidified with HCl before the AAS analysis. Unbound platinum carried over into the precipitate was corrected for by adding cisplatin to cell extracts immediately before adding the TCA.

2.9. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Cells were harvested and washed twice with PBS. Total RNA was extracted from pellets of 1×10^6 cells using the 'RNeasy Mini' Kit (Qiagen, West Sussex, UK). All cDNA syntheses were carried out using the SUPERSCRIPT^{TM} Preamplification System for First Strand cDNA synthesis (GibcoBRL, Life Technologies). For each amplification, 10% of the cDNA reaction mixture was used in a total reaction volume of 50 μl (1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 0.2 μM of each primer, 2U Taq-DNA polymerase and 1× PCR reaction mix (GibcoBRL, Life Technologies)). Depending on the primers being used, a total of 22–31 cycles of PCR, (94 °C/30 s, 60 °C/30 s and 72 °C/90 s) were performed. The primer sequences for *hTERT*, telomerase-associated protein (*TEP1*), and *hTR* were those previously reported by Takakura and colleagues [25]. *hTERT* and *TP1.1* cDNAs were amplified

Table 1
Concentrations of drugs that caused 50% growth inhibition[a]

Cell line	Cisplatin (nM)	Melphalan (nM)	Doxorubicin (nM)
Susa CP	797±101	725±158	13.6±2.3
833K	324±31	763±106	19.3±3.6

[a] Following a 4-h drug exposure, cells were incubated for 5 days and then assayed using the sulphorhodamine B (SRB) method.

using 28 cycles and *hTR* was amplified using 25 cycles. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cDNA was amplified using primers previously described in Ref. [26] using 22 PCR cycles. The PCR products were analysed on a 2% (w/v) agarose gel containing ethidium bromide (0.25 μg/ml) and viewed under ultraviolet (UV) transillumination.

3. Results

3.1. Telomerase activity in Susa CP and 833K cells treated with cytotoxic drugs

Exponentially growing Susa CP cells were incubated for 4 h with cytotoxic drugs, and telomerase activity was measured after further incubation in drug-free medium. Quantification of band intensities at 1 or 10× IC_{50} concentrations showed no significant loss of telomerase activity at 20 or 44 h following drug exposure (data not shown). At higher drug concentrations (100× IC_{50}), a significant reduction in telomerase activity was observed in the cisplatin-treated cells (mean ± standard deviation (S.D.) = 56 ± 24%, $P < 0.0001$) at 20 h (Fig. 1a), whereas no reduction in telomerase activity was observed at 20 h in melphalan- or doxorubicin-treated cells. A cisplatin-specific loss of telomerase activity at 20 h was in accordance with the data of Burger and colleagues [17]; however, by extending the post-drug incubation time to 44 h, the loss of telomerase activity was found not to be entirely specific to cisplatin. At this time point, although a further loss of activity was observed with cisplatin to 9 ± 5% of the control ($P < 0.0001$), telomerase activity had also declined markedly after melphalan exposure (Fig. 1b). However, even at 44 h, no loss of telomerase activity was observed in doxorubicin-treated cells (Fig. 1c). None of the drug treatments induced a significant loss of activity at 6 h (Fig. 1). As with the Susa CP cells, telomerase activity in the 833 K cells following a 4-h exposure to 100× IC_{50} concentrations of cisplatin showed a significant reduction at 20 and 44 h (Fig. 1d and data not shown). Again, incubation for more than 6 h following exposure to 100 μM cisplatin was required before significant loss of telomerase was observed (Fig. 1d). Overall, a differential loss of telomerase activity was observed following exposure to cisplatin, melphalan and doxorubicin.

3.2. Comparison of the levels of apoptosis and telomerase activity following exposure of Susa CP and 833K cells to cisplatin, melphalan or doxorubicin

The levels of apoptosis following drug treatment were determined in Susa CP cells using nuclear morphology to measure apoptosis. Following 4-h treatments with 100× IC_{50} concentrations of cisplatin, melphalan or doxorubicin, the frequencies of cells with apoptotic morphology increased at similar rates (Fig. 2a). Drug-induced changes in both the telomerase activity and levels of apoptosis (by annexin V labelling and nuclear morphology) were then studied in both Susa CP and 833K using samples collected from the same cultures. The proportion of Susa CP cells that had undergone apoptosis at 20 h after a 4-h exposure to 100× IC_{50} concentrations of cisplatin, melphalan or doxorubicin were similar (Fig. 2b). However, in the same cultures telomerase activity at 24 h following treatment with cisplatin was reduced whilst after treatment with melphalan or doxorubicin, telomerase activity was the same as in control cells (Fig. 2b). Following exposure to 100× IC_{50} concentrations of each drug, higher proportions of 833 K cells exhibited apoptotic morphology compared with Susa CP cells. In 833 K cells, both cisplatin and melphalan caused a significant loss of telomerase activity

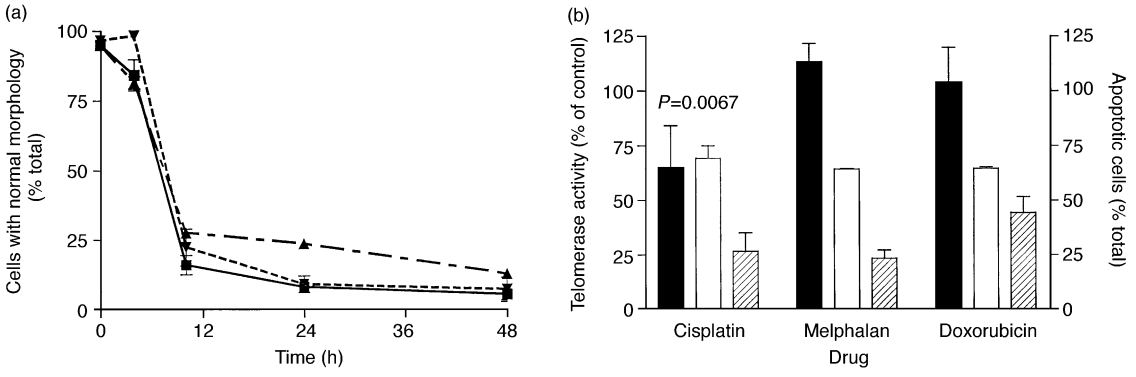

Fig. 2. (a) Decline in the number of Susa CP cells showing normal nuclear morphology (assessed by staining with Hoechst 33258 dye) following a 4-h exposure to; –■– cisplatin (100 μM), - -▲- - melphalan (100 μM) or -·▼·- doxorubicin (1.3 μM). In control cultures, the levels of cells with normal morphology was >95% at all time points. Each point represents the mean ± standard deviation (S.D.) of three independent experiments. (b) Levels of telomerase activity and frequencies of apoptotic cells measured in the same cultures of Susa CP cells following a 4-h exposure to cisplatin, melphalan or doxorubicin (100× the IC_{50}) and 20 h in drug-free medium Solid bars: telomerase activity as percentage of control cells (P = probability that mean telomerase activity <100%). Apoptotic cells were scored by nuclear morphology (open bars) and by annexin V staining (hatched bars).

($P < 0.0001$ and $P = 0.002$, respectively), whereas doxorubicin had no significant effect (Fig. 3a and b). As observed in Susa CP cells cisplatin, melphalan and doxorubicin caused similar high levels of apoptosis in 833 K cells (Fig. 3b). On the basis of these data, it was concluded that the differential loss of telomerase activity observed in Susa CP and 833K cells following exposure to cisplatin, melphalan and doxorubicin was not related to the levels of drug-induced apoptosis. Alternative mechanisms for the loss of telomerase activity following cisplatin treatment were therefore investigated.

3.3. Direct effect of cisplatin on telomerase activity in Susa CP and 833 K cell extracts

To investigate the possibility that cisplatin-induced loss of telomerase was due to a direct reaction with the enzyme, cell extracts were incubated with cisplatin before measuring telomerase activity. Ideally, Susa CP and 833K cell extracts would have been exposed to cisplatin under the conditions used for whole cells (i.e. 100 μM×4 h, 37 °C). However, this was not feasible because of the instability of telomerase in cell extracts under these conditions. Instead, Susa CP and 833K cell extracts were incubated with cisplatin (1000 or 100 μM) for 30 min at 30 °C, and in the absence of cisplatin there was no detectable loss of enzyme activity. Results from three replicate experiments (Table 2) show that cisplatin caused a small, but not significant, reduction in the telomerase activity of the Susa CP extracts, and no detectable loss in the 833K extracts. As a measure of the overall extent of platinum reactivity in these experiments, the amounts of platinum that became bound to macromolecules in the cell extracts were determined (Table 2) for comparison to equivalent measurements on drug-treated cells. The amount of platinum bound to macromolecules in the extracts from Susa CP and 833K cells exposed to 100 μM cisplatin for 4 h were 19±2 pg Pt/μg and 51±5 pg Pt/μg protein, respectively. Thus, for both cell lines the level of platinum that bound to macromolecules following incubation of cell extracts, with even the lowest concentration of cisplatin used, were several-fold higher than the levels which occurred during the drug treatment of cells. Since these high levels of binding of platinum to macromolecules in cell extracts were not associated with a significant loss of

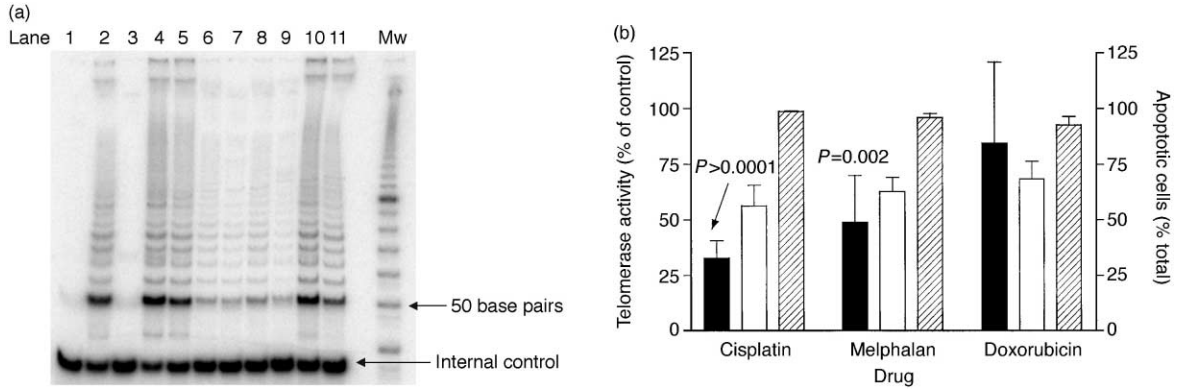

Fig. 3. (a) Representative result from a TRAP*eze* assay for telomerase activity in 833K cells following a 4-h exposure to cisplatin, melphalan or doxorubicin (100× the IC_{50}) and 20 h in drug-free medium. Lane 1: lysis solution only; lane 2: TRAP*eze* quality control cell lysate (HeLa cells); lane 3: heat treated extract of control cells (equivalent to lane 4); lanes 4 and 5: control cells; lanes 6 and 7: cells treated with cisplatin; lanes 8 and 9: cells treated with melphalan; lanes 10 and 11: cells treated with doxorubicin. Protein concentration per assay were 0.3 μg (lanes 3, 4, 6, 8 and 10) and 1.0 μg (lanes 5, 7, 9 and 11). Mw: molecular weight marker with 10 base pair increment. (b) Levels of telomerase activity and apoptotic cells measured in the same cultures of 833K cells following a 4-h exposure to cisplatin, melphalan or doxorubicin (100× the IC_{50}) and 20 h in drug-free medium. Solid bars: telomerase activity as percentage of control cells (P = probability that mean telomerase activity <100%). Apoptotic cells were scored by nuclear morphology (open bars) and by annexin V staining (hatched bars). IC_{50}, drug concentrations causing 50% growth inhibition.

Table 2
Telomerase activity and the amount of platinum bound to macromolecules (expressed per μg of protein) in Susa CP and 833K cell extracts following either a 1000 μM or 100 μM cisplatin treatment for 30 min at 30 °C[a]

	Telomerase activity and platinum bound to macromolecules in cell lysates			
	Susa CP		833K	
Drug:	1000 μM cisplatin	100 μM cisplatin	1000 μM cisplatin	100 μM cisplatin
Telomerase activity	67±21%	85±12%	98±8%	97±6%
pg Pt/μg protein	1200±200	70±40	2300±700	150±40

[a] The mean±standard deviation (S.D.) of pg Pt/μg protein was calculated using atomic adsorption spectrometer (AAS) from three replicate experiments. The results are corrected for any carry over of platinum.

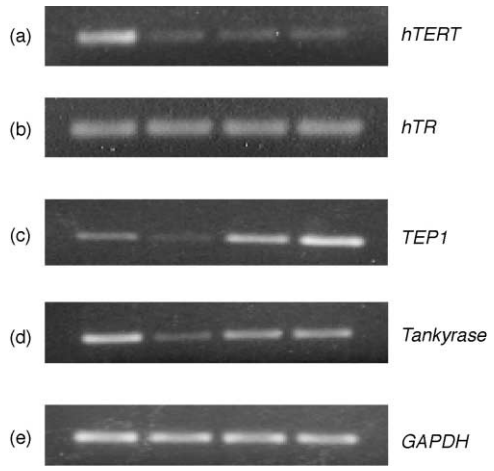

Fig. 4. Representative reverse transciptase-polymerase chain reaction (RT-PCR) results from three separate experiments for levels of (a) mRNA for the catalytic subunit component of telomerase (*hTERT*); (b) the RNA component of telomerase (*hTR*): (c) mRNA for the telomerase-associated protein (*TEP1*); (d) mRNA for *tankyrase* and (e) mRNA levels for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in Susa CP cells exposed to 100× IC_{50} concentrations of cisplatin, melphalan or doxorubicin for 4 h followed by 20 h in drug-free medium. Lane 1: control cells exposed to solvent only (1% (v/v) dimethylsulphoxide (DMSO); lane 2: cells treated with 100 μM cisplatin; lane 3: cells treated with 100 μM melphalan; lane 4: cells treated with 1.3 μM doxorubicin.

telomerase activity, the results were not consistent with the cisplatin-induced loss of telomerase activity in Susa CP and 833K cells being a direct effect of the drug on the enzyme.

3.4. Effect of drug exposure on the expression of telomerase components

To evaluate possible effects of cisplatin on the transcription of genes coding for telomerase, and associated components, the effects of cisplatin, melphalan and doxorubicin (4 h at 100× IC_{50} concentrations) on levels of the telomerase catalytic subunit (*hTERT*) mRNA, RNA component (*hTR*), *TEP1* mRNA and the telomeric protein, *tankyrase* mRNA were investigated in exponentially growing Susa CP cells by RT-PCR. Three separate experiments each showed that all three drugs reduced the level of *hTERT* mRNA to a similar extent at 24 h, compared with the level of *hTERT* mRNA in untreated cells (Fig. 4a). No reduction in the levels of the telomerase RNA component at 24 h after cisplatin, melphalan or doxorubicin treatment was observed (Fig. 4b). Interestingly, cisplatin, melphalan and doxorubicin treatment showed differential effects on *TEP1* and *tankyrase* mRNA at 24 h (Fig. 4c and d). In cisplatin-treated cells, there was a decrease in *TEP1* and *tankyrase* mRNA compared with control cells, whereas the levels of *TEP1* mRNA following doxorubicin treatment increased compared with control cells. The efficiency of the cDNA synthesis from each sample was estimated by PCR with *GAPDH*-specific primers (Fig. 4e). Overall, the expression of essential telomerase components necessary for activity (*hTERT* and *hTR*) was not related to the telomerase activity in Susa CP cells following drug-induced cell killing.

4. Discussion

The aim of the studies described in this paper was to determine the utility of telomerase activity as a marker of cytotoxic drug-induced apoptosis, with a view to using telomerase activity as an endpoint in chemosensitivity studies. Previous studies [11–13,17–19] have provided conflicting evidence of the relationship between telomerase activity and cytotoxic drug-induced activity or apoptosis, and to address this question the current investigation compared the effects of three cytotoxic drugs (cisplatin, melphalan and doxorubicin) in two cell lines (Susa CP and 833K) using two methods to measure apoptosis (nuclear morphology and annexin V staining). The three cytotoxic drugs used all produced similar levels of apoptosis at equal multiples of growth inhibitory concentrations, and induced apoptosis over a similar time course. Cisplatin did induce loss of telomerase activity in Susa CP cells at 20 and 44 h, but not 6 h, after a 4-h exposure, and only when a concentration equivalent to approximately 100× the IC_{50} (i.e. 100 μM cisplatin) was used. Loss of telomerase activity in the Susa CP cells was not observed following a 4-h exposure to cisplatin at either 1 or 10× IC_{50} concentrations. Similar results were obtained at 6 and 20 h in the 833 K cells with telomerase loss only being observed following exposure to a cisplatin concentration equivalent to approximately 100× the IC_{50} (100 μM cisplatin). Following exposure to melphalan, loss of telomerase activity was again only observed following exposure to a 100× IC_{50} concentration in both Susa CP (at 44 h, Fig. 1b) and 833K cells (at 20 h, data not shown). Most notably, exposure to 100× IC_{50} concentrations of doxorubicin failed to induce loss of telomerase activity in either cell line at any of the time points studied, despite the presence of similar levels of apoptosis to those seen following cisplatin or melphalan treatment. On the basis of these data, we concluded that loss of telomerase activity is not consistently associated with cytotoxic drug-induced apoptosis. Furthermore, even when loss of telomerase activity is observed highly toxic drug concentrations are required to demonstrate such an effect.

The conclusions of this study are consistent with those of Burger and colleagues [17] in relation to the differential effects of the three drugs studied on telomerase activity in Susa CP cells, and to the results of Lin and colleagues [19] and Multani and colleagues [18] concerning the lack of a relationship between cytotoxic drug-induced growth inhibition/apoptosis and loss of

telomerase activity. However, the results presented here are not in accord with those of Faraoni and colleagues [11], who reported a relationship between growth inhibition and telomerase loss. As suggested by Akiyama and colleagues [16], the different experimental protocols and cell lines used may explain the discrepancies in the results of the different studies, a suggestion which reinforces the restricted utility of telomerase loss as a basis for chemosensitivity testing.

In both of the testicular teratoma cell lines studied here, cisplatin treatment produced loss of telomerase activity, albeit only after exposure to supra-toxic concentrations. As discussed above, the lack of a clear relationship to apoptosis and the differential effects of cisplatin, melphalan and doxorubicin suggests that the loss of telomerase activity is due to a compound-specific mechanism as opposed to a non-specific effect of cell death. Direct reaction with the enzyme or reduced telomerase RNA component (*hTR*) levels were excluded as the mechanism underlying cisplatin-induced telomerase loss, the latter observation being consistent with the results of Akiyama and colleagues [16]. However, Akiyama and colleagues [16] did report that loss of telomerase activity was associated with reduced catalytic domain (*hTERT*) mRNA levels, whereas in the current study reduced *hTERT* mRNA was produced by all three drug treatments despite the lack of any effect of doxorubicin on telomerase activity over the time period studied.

In conclusion, the present results for testicular teratoma together with other reports [18,19] indicate that loss of telomerase activity is not a universal marker of cytotoxic drug-induced apoptosis. Thus, its utility will have to be established for any given cell type. In addition, loss of telomerase was only produced by highly toxic drug concentrations. Treatment with cisplatin at high concentrations did result in the loss of telomerase activity in both of the cell lines studied, and the mechanism was not related to direct inactivation of the enzyme, or reduced *hTR* RNA levels. The usefulness of telomerase activity as a reliable indicator of chemosensitivity in other cell types requires further investigation.

Acknowledgements

This work was supported by the Cancer Research Campaign. We would like to thank Professor J.W.R. Masters for providing the cell lines.

References

1. McClintock B. The behavior in successive nuclear divisions of a chromosomes broken at meiosis. *Genetics* 1939, **25**, 405–416.
2. Blackburn BH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosome ribosomal RNA genes in tetrahymena. *J Mol Biol* 1978, **120**, 33–53.
3. GoldStein S. Replicative senescence: the human fibroblast comes of age. *Science* 1990, **249**, 1129–1133.
4. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonuleoprotein enzyme with two kinds of primer specificity. *Cell* 1987, **51**, 887–898.
5. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. *Nature* 1989, **337**, 331–337.
6. Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF. Telomerase activity in human breast tumours. *J Natl Cancer Inst* 1996, **17**, 116–122.
7. Counter MN, Hirte HW, Bacchetti S, Harley CB. Telomerase activity in human ovarian carcinoma. *Proc Natl Acad Sci USA* 1994, **91**, 2900–2904.
8. Kim NW, Piatyszek MA, Prowse KR, *et al*. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994, **266**, 2011–2014.
9. Hahn WC, Counter C, Lundberg AS, Beijersbergen RL, Brooks MW, & Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature* 1999, **400**, 464–469.
10. Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyzek MA, Shay JW. Correlating telomerase activity levels with human neuroblastoma outcomes. *Nat Med* 1995, **1**, 249–255.
11. Faraoni I, Turriziani M, Masci G, *et al*. Decline in telomerase activity as a measure of tumour cell killing by antineoplastic agents in vitro. *Clin Cancer Res* 1997, **3**, 579–585.
12. Faraoni F, Graziani G, Turriziani M, *et al*. Suppression of telomerase activity as an indicator of drug-induced cytotoxicity against cancer cells: in vitro studies with fresh human tumor samples. *Lab Invest* 1999, **79**, 993–1005.
13. Mese H, Ueyama YAS, Nakayama S, Sasaki A, Hamakawa H, Matsumura T. Inhibition of telomerase activity as a measure of tumour cell killing by cisplatin in a squamous cell carcinoma cell line. *Chemotherapy* 2001, **47**, 136–142.
14. Asai A, Kiyozuka Y, Yoshida R, Fujii T, Hioki K, Tsubura A. Telomere length, telomerase activity and telomerase RNA expression in human esophageal cancer cells: correlation with cell proliferation, differentiation and chemosensitivity to anticancer drugs. *Anticancer Res* 1998, **18**, 1465–1472.
15. Park KH, Rha SY, Kim CH, *et al*. Telomerase activity and telomeric lengths in various cell lines: changes of telomerase avtivity can be another method for chemosensitive evaluation. *Int J Oncol* 1998, **13**, 489–495.
16. Akiyama M, Horiguchi-Yamada H, Saito S, *et al*. Cytostatic concentrations of anticancer agents do not affect telomerase activity of leukaemic cells in vitro. *Eur J Cancer* 1999, **35**, 309–315.
17. Burger AM, Double JA, Newell DR. Inhibition of telomerase activity by cisplatin in human testicular cancer cells. *Eur J Cancer* 1997, **33**, 638–644.
18. Multani AS, Furlong C, Pathak S. Reduction of telomeric signals in murine melanoma and human breast cancer cell lines treated with 3′-azido-2′-3′-dideoxythymidine. *Int J Oncol* 1998, **13**, 923–925.
19. Lin Z, Lim S, Viani MA, Sapp M, Lim MS. Down-regulation of telomerase activity in malignant lymphomas by radiation and chemotheraputic agents. *Am J Pathol* 2001, **159**, 711–719.
20. Walker MC, Povey S, Parrington M, Riddle PN, Knuechel R, Masters RW. Development and characterization of cisplatin-resistant human testicular and bladder tumour cell lines. *Eur J Cancer* 1990, **26**, 742–747.
21. Bronsen DL, Andrews PW, Solter D, Cervenka J, Lange PH, Fraley EE. Cell line dervied from a metastasis of a human testicular germ cell tumor. *Cancer Res* 1980, **40**, 2500–2506.
22. Holt SE, Norton JC, Wright WE, Shay JW. Comparison of the telomeric repeat amplification protocol (TRAP) to the new TRAP-eze telomerase detection kit. *Methods Cell Sci* 1996, **18**, 237–248.

23. Skehan P, Storeng R, Scudiero DA. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990, **82**, 1107–1112.
24. Glazal-Asward S, Calvert AH, Newell DR. A single-sample assay for the estimation of the area under the free carboplatin plasma concentration versus time curve. *Cancer Chemother Pharmacol* 1996, **37**, 429–434.
25. Takakura M, Kyo S, Kanaya T, Tanaka M, Inoue M. Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. *Cancer Res* 1998, **58**, 1558–1561.
26. Nakamura TM, Morin GB, Chapman KB, *et al*. Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997, **277**, 955–959.