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Original Paper

Inhibition of Telomerase Activity by Cisplatin in Human Testicular Cancer Cells

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Telomerase, a ribonucleoprotein, elongates and/or maintains telomeres by adding TTAGGG tandem repeat sequences using the RNA component of the enzyme as a template. Enzyme activity appears to be associated with cell immortalisation and malignant progression as telomerase activity has been found in the majority of human tumours, but not in most somatic cells or tissues. Telomerase inhibition has, therefore, been proposed as a novel and potentially selective target for therapeutic intervention. Since telomeric tandem repeats as well as the human telomerase RNA component (hTR) and its gene are guanosine-rich, we examined whether the sequence specific, G–Pt–G, cross-linking agent cisplatin is capable of inhibiting telomerase activity. The TRAP assay was used to measure telomerase activity in cisplatin treated cell extracts and RT-PCR strategies used to examine hTR expression after drug exposure. Cisplatin reduced telomerase activity in a specific and concentration-dependent manner in human testicular tumour cells, whilst doxorubicin, bleomycin, methotrexate, melphalan and transplatin had no effect. It is proposed that telomerase inhibition might be a component of the efficacy of cisplatin in the treatment of testicular cancer. © **1997 Elsevier Science Ltd. All rights reserved.**

Key words: telomerase activity, cisplatin, testicular cancer, growth inhibition, telomerase inhibition, cross-linking, human telomerase RNA component

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INTRODUCTION

TELOMERASE IS believed to play a key role in the stabilisation and maintenance of chromosome ends by adding the tandem repeat sequence TTAGGG in vertebrate organisms [1]. Telomere shortening down to a critical length leads to cell senescence and death [2, 3]. Thus, transformed cells are thought to escape mitotic crisis by activating the ribonucleoprotein enzyme, telomerase [4–6]. Several studies have shown that telomerase activity is present in the majority of tumour tissues and can be linked to acquisition of malignancy in certain tumour model systems [4, 7, 8]. Based on these findings, it has been proposed that inhibition of telomerase activity could have significant potential as an anticancer therapy [9, 10]. Some inhibitors of retroviral reverse transcriptase have been reported to alter or reduce telomerase activity and induce telomere shortening in *tetrahymena* and human lymphoid cells [11, 12], and it has been further demonstrated that antisense oligonucleotides and peptide nucleic acids, which target the telomerase RNA component (hTR), can inhibit telomerase activity in cell-free systems [13, 14]. However, inhibition of telomerase activity and telomere shortening have not been consistently linked to growth inhibition in tumour cells.

Targeting the strikingly guanosine-rich regions of the telomeric tandem repeat sequences, the hTR component and its gene [13], might represent another potential antitelomerase strategy. Certain standard anticancer agents are known to have sequence selectivity for specific nucleotides in their mode(s) of action [15]. Particularly, platinum complexes have been described as inflicting DNA damage by recognising defined nucleotide patterns [15]. It is understood that the cross-linking agent, cisplatin, forms primarily intrastrand lesions with more than 50% of cross-links resulting in G–Pt–G adducts [16, 17]. Cisplatin is an essential component of combination chemotherapy in the treatment of testicular

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cancers, which are curable in over 80% of all cases [18], although the precise mechanism underlying cisplatin sensitivity of these tumours remains unclear [16, 17, 19]. Here, we investigated whether cisplatin treatment could cause telomerase inhibition in correlation with growth arrest by using well-characterised testicular cancer cell lines as experimental systems [16, 17, 19], and whether inhibition of telomerase activity might contribute to the unique cisplatin sensitivity of germ cell derived tumours. Other DNA damaging agents, which act by different and/or similar mechanisms, were tested in comparison with cisplatin to examine the specificity of telomerase inhibition.

MATERIALS AND METHODS

Drugs

The cisplatin (CDDP) used was a gift from Dr L.R. Kelland (Institute of Cancer Research, Sutton, Surrey, U.K.) and transplatin (tDDP) from Johnson Matthey (Reading, Berks, U.K.). All other drugs were purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, U.K.). Cisplatin and transplatin stock solutions were prepared in *N,N*-dimethylacetamide (DMA), melphalan (MEL) was dissolved in dimethyl sulphoxide (DMSO); doxorubicin (DOX), bleomycin (BLEO) and methotrexate (MTX) were used as aqueous solutions.

Cell culture and drug treatment

The testicular cancer cell lines, F9 (mouse), 833K, Susa and Susa CP (all human), were kindly provided by Dr J.R.W. Masters (University College, London, U.K.) and have been extensively characterised [16, 17, 19].

For short-term exposure to the drugs (4 h), approximately 1.5×10^6 Susa CP cells in exponential growth were seeded in 10 ml RPMI 1640 medium (supplemented with 2 mM L-glutamine and 10% fetal calf serum, 25 cm^2 flasks, Gibco BRL, Paisley, U.K.) 24 h prior to drug treatment. Drugs were added in 10 ml of fresh RPMI 1640 to final concentrations (see Table 1 and Figures). Control cells were treated with DMA or DMSO instead. Cells were incubated for 4 h at 37°C/5% CO_2 humidified atmosphere and the drug was then removed by washing the cells twice with Hank's Balanced Salt Solution (HBSS, Gibco BRL, Life Technologies Ltd, Paisley, U.K.). Drug exposure was followed by an additional 20 h incubation in drug-free medium to allow the turnover of cellular proteins before extraction of telomerase activity. For 100 μM CDDP, telomerase was also extracted directly after the 4 h exposure.

For continuous exposure to the drugs (6 days), drug or solvent only (control cells) was added to 5×10^6 exponentially growing Susa CP cells at various concentrations (see Table 1 and Figures), and the cells were incubated for 6 days at 37°C/5% CO_2. After this incubation period, cells were washed twice with ice-cold HBSS and the enzyme activity extracted.

Table 1. Cytotoxicity data

Drug	Susa CP IC_{50}* ± S.E. [μM]	TRAP assay concentration for 4 h exposure** [μM]
Cisplatin (CDDP)	1.90 ± 0.25	100
Melphalan (MEL)	1.55 ± 0.45	100
Transplatin (tDDP)	3.10 ± 0.10	150

*Concentrations of cytotoxic agents causing 50% growth inhibition (IC_{50}) after 6 days continuous exposure to drug determined by MTT assay [27]. Values are given for the Susa CP testicular cancer cell line (3-fold cisplatin resistant in comparison to parent Susa cell line) [16, 17]. The cisplatin concentration necessary to achieve total growth inhibition in 6 days continuous exposure experiments was found to be 25 ± 1 μM and the IC_{50} for cisplatin after 4 h short-term exposure was 16 ± 1 μM.

**TRAP assay concentrations represent those used for a 4 h exposure followed by subsequent incubation for 20 h in drug-free medium

Telomerase assay

The highly sensitive telomeric repeat amplification protocol (TRAP assay) was used to measure telomerase activity as described previously [4].

Protein/enzyme extraction and estimation

To extract telomerase activity from the cell pellets, cells were washed in telomerase washing buffer (10 mM (*N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethanesulphonic acid (HEPES)-KOH pH 7.5, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM 1,4-dithiothreitol (DTT) [4]) followed by cell lysis with the CHAPS-based (3-[(3-cholamido propyl) dimethyl ammonio]-1-propane-sulphonate) telomerase lysis buffer, (10 mM Tris-HCl pH 7.5, 1 mM $MgCl_2$, 1 mM ethylene glycol-bis-(β-aminoethylether)-*N,N,N,N*-tetracetic acid (EGTA), 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 5 mM 2-mercaptoethenol (2-ME), 0.5% CHAPS, 10% glycerol [4]). Homogenates were kept on ice for 30 min and then centrifuged for 30 min at 417 000 *g*/4°C (Beckman, Optima TL Ultracentrifuge). The supernatant was removed carefully and stored at −80 °C. Total cellular protein was determined by the method of Bradford [20] using the BioRad Protein Assay (BioRad Laboratories, Munich, Germany). A range of 10.0–0.001 μg total cellular protein extract was employed for the TRAP reaction. Optimal protein concentrations for the cell lines used in this study, which just saturate the PCR (polymerase chain reaction), but do not inhibit the Taq-DNA polymerase [21] were found to be 1.0–0.1 μg. To allow comparison of telomerase activity in treated or untreated cells, the same amounts of total cellular protein (0.1, 0.01 and 0.001 μg) were assayed under the same conditions. These 10-fold dilutions were freshly prepared in sterile diethyl pyrocarbonate-treated water just prior to addition to the PCR reaction mixture. Total cellular protein (0.1 μg) of the Susa CP cell line, which gives a very prominent and extensive ladder signal, functioned as positive control and a lysis buffer blank was used as the negative control for each set of probes examined.

TRAP assay

The CX primer, 5'-CCCTTACCCTTACCCTTACCCTAA-3', was used as downstream PCR primer and was initially separated from the rest of the reaction by a wax barrier (Ampliwax, Perkin Elmer, Branchburg, New Jersey, U.S.A.), whilst the TS primer 5'-AATCCGTCGAGCAGAGTT-3', necessary for the telomerase extension reaction, was contained in the 50 μl reaction mix (20 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween-20, 1 mM EGTA, 50 μM deoxynucleotide-5'-triphosphate (dNTP), 0.1 μg TS primer, 1 μg T4 gene 32 (T4g32) protein, 5 μg bovine serum albumin

(BSA), 2 U Taq-DNA polymerase and 2 μCi [α-^{32}P]-dCTP) [4]. Cell lysates were added to the PCR reagents and incubated for 30 min at room temperature to allow telomerase, if present, to extend the TS oligonucleotide. After heating the samples at 90°C for 90 sec, telomerase products were amplified in 31 PCR cycles at 94°C for 30 sec, 50°C for 30 sec and 72°C for 45 sec.

The whole sample (approximately 50 μl) was loaded on to a 10% acrylamide non-denaturating gel and resolved by electrophoresis at 155 V for 5–6 h in 0.5× Tris-borate EDTA (TBE) buffer. The average gel exposure duration to film was 20 h at −80 °C.

Data of three independent experiments were collected and representative data are shown.

RT-PCR

Direct effects of cytotoxic agents on the expression of the human telomerase RNA component (hTR) was examined by an RT-PCR (reverse transcription-PCR) technique. Susa CP cells were prepared and treated with drug or vehicle for 4 h, washed and incubated for an additional 20 h as described above. Total RNA was extracted from cells following the RNAzol B (Tel-Test, Friendswood, Texas, U.S.A.) method according to the manufacturers instructions. RNA (0.05 μg) was then reverse transcribed into single-stranded cDNA with AMV reverse transcriptase and random primers using the first-strand cDNA synthesis RT-PCR kit from Boehringer (Boehringer Mannheim Ltd, Lewes, U.K.). For the PCR reaction, hTR sequence specific TRC3 primers, described by Feng and associates [13] (see below) were used to amplify telomerase RNA component cDNA. PCR products were separated on a 1.5% agarose gel (MetaPhor agarose, Flowgen, Lichfield, U.K.; in 0.5× TBE buffer, 75 V, 3 h) and stained with ethidium bromide. Beta-actin was amplified as an internal standard using 5′-TCATACTCCTGCTGCTTGCTGATCC-3′ as antisense

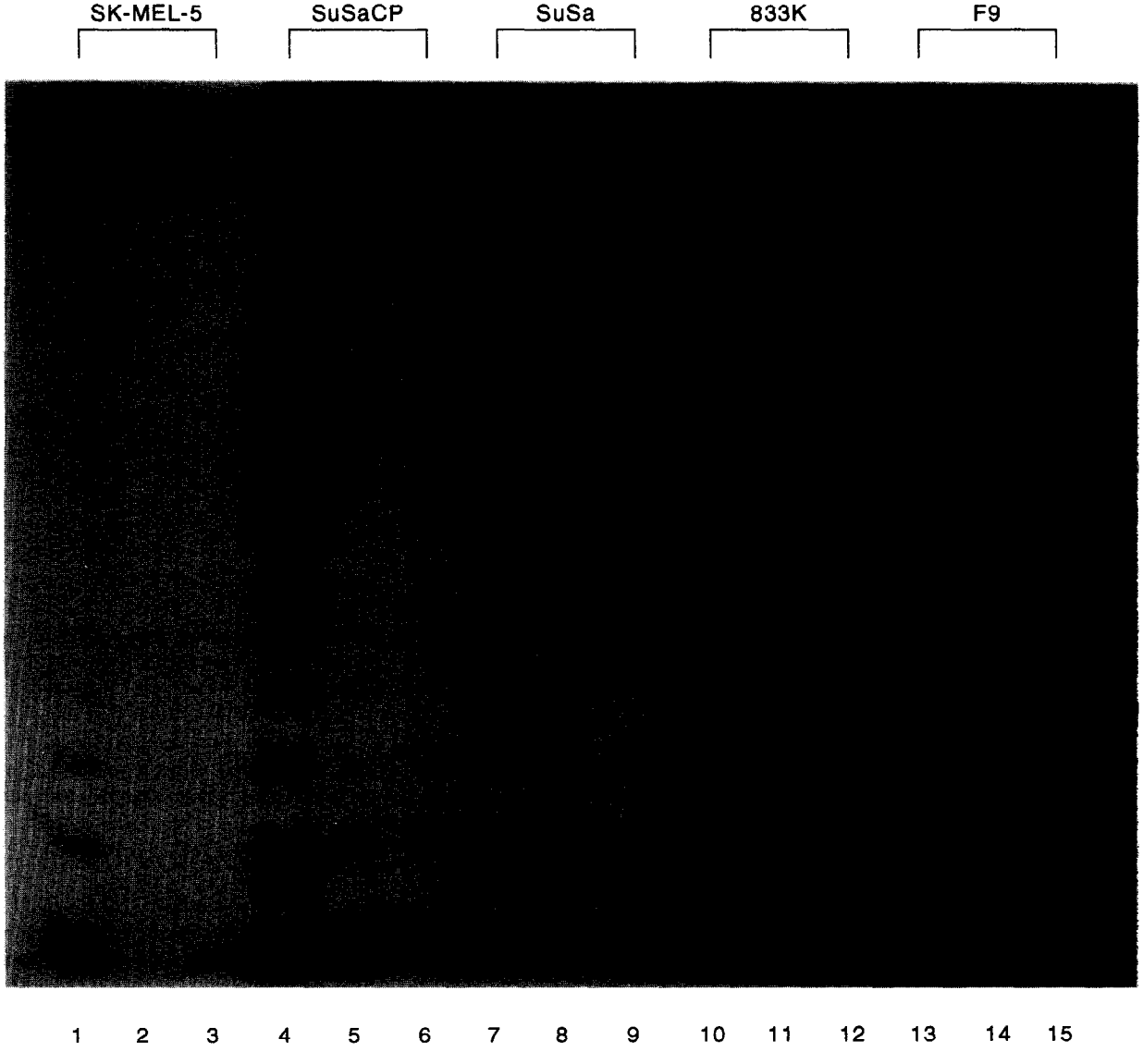

Figure 1. Telomerase activity in testicular cancer cell lines. Compared are telomerase levels of three human testicular cancer cell lines, Susa CP, Susa and 833K, with a murine testicular cancer cell line, F9, and a melanoma cell line, SK-MEL-5. Three concentrations of total cellular protein for each cell line were assayed to attempt quantitation of telomerase activity. lanes 1, 4, 7, 10 and 13 represent telomerase activity in 0.1 μg total cellular protein; lanes 2, 5, 8, 11 and 14 show 0.01 μg and lanes 3, 6, 9, 12 and 15, 0.001 μg protein

and 5′-CCACGAAACTACCTTCAAC-TCC-3′ as sense primer.

TRC3 primers

5′-CTAACCCTATCTGAGTTGGGCGTA-3′
and
5′-CAACGGACAGACAGCAGCTGACAT-3′

The identity of the TRC3 primer product as a part of the human telomerase RNA component was confirmed by digesting the resulting cDNA fragment with Hae III and Eco RI (Sigma Chemicals, Poole, U.K.) restriction enzymes.

RESULTS

The telomeric repeat amplification protocol (TRAP assay) was used [4] to measure telomerase activity in testicular cancer cell lines and to examine the effect of CDDP and other DNA interactive agents on enzyme levels. Testicular tumour cells readily express telomerase activity as depicted in Figure 1. If extracted and assayed under the same conditions, other cell lines such as SK-MEL-5 had less telomerase activity. The testicular cancer cell lines Susa and Susa CP, the latter slightly (3-fold) cisplatin resistant in comparison to the parent cell line Susa, were chosen for detailed evaluation of drug effects. The Susa CP cell line was used to assess the effect of cisplatin on telomerase activity after short-term (Figure 2) and continuous exposure (Figure 3) to the drugs because it is well characterised and in our hands was the best growing line. For 6 days continuous treatment, concentrations resulting in 50% growth inhibition and total growth inhibition were used, whilst for 4 h exposure, lethal drug concentrations were added (Table 1, Figures 2 and 4). Inhibition of telomerase activity by cisplatin was observed in Susa CP cells, which had been exposed to 100 μM cisplatin (IC_{100}) for 4 h and had been incubated for an additional 20 h in drug-free medium following the 4 h drug treatment. However, telomerase activity was not

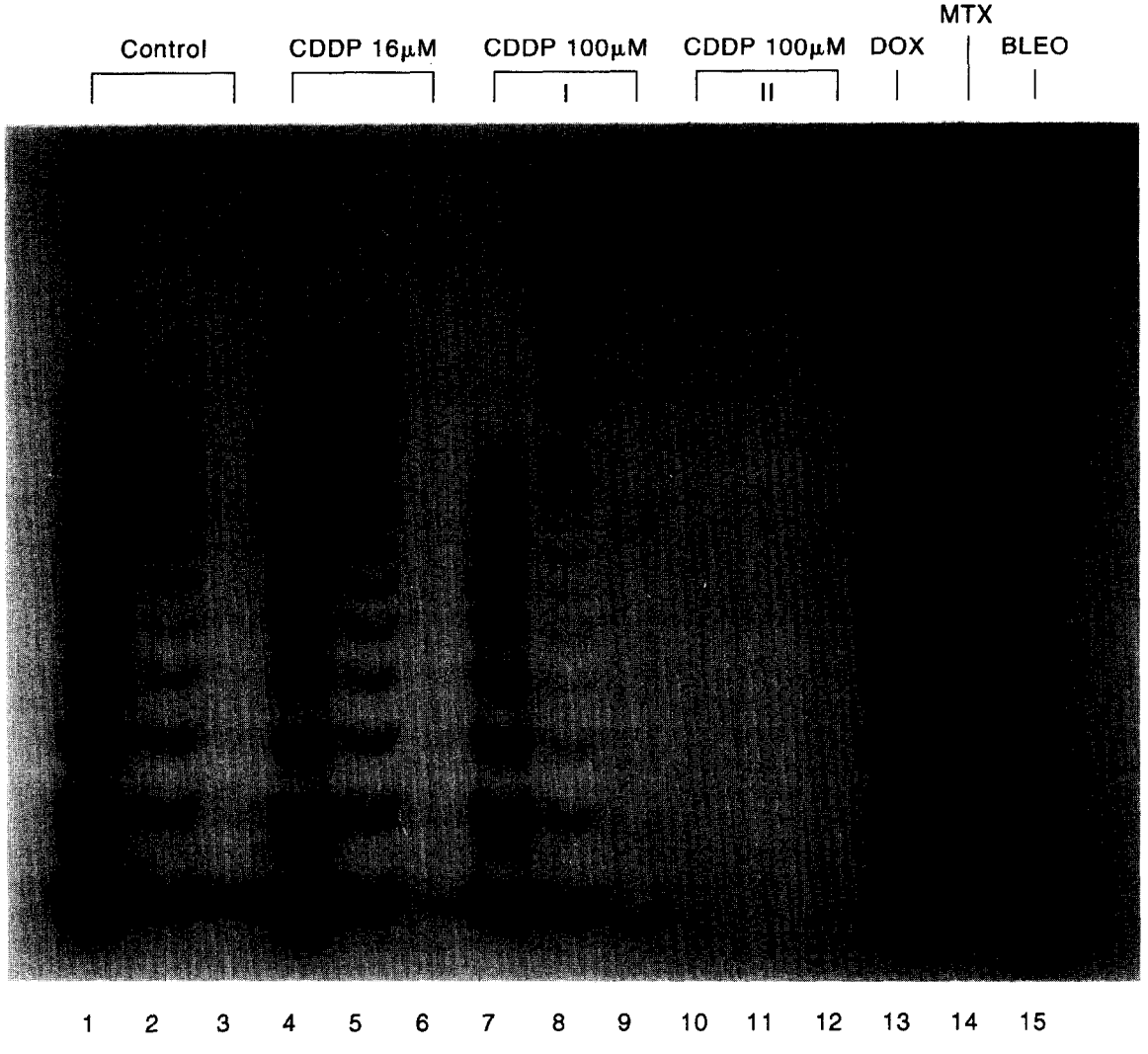

Figure 2. Telomerase activity in Susa CP testicular cancer cell lines after 4 h exposure to cytotoxic agents. Effects on enzyme activity in cells treated with cisplatin at IC_{50} (16 μM lanes 4–6) or lethal concentrations (100 μM; I, lanes 7–9 telomerase activity assayed without incubation in drug-free medium; II; lanes 10–12 telomerase activity assayed in cells incubated for 20 h in drug-free medium after 4 h exposure to 100 μM cisplatin). For comparison, Susa CP cells were also exposed to supralethal concentrations of DOX (1.5 μM, IC_{50} for 6 days continuous exposure 3 nM [16]), MTX (30 μM; IC_{50} for 6 days continuous exposure 55 nM [16]) and BLEO (5.5 μM; IC_{50} for 6 days exposure to drug 5.5 nM [16]). Lanes 1 (0.1 μg), 2 (0.01 μg) and 3 (0.01 μg protein assayed) represent vehicle only treated Susa CP cells

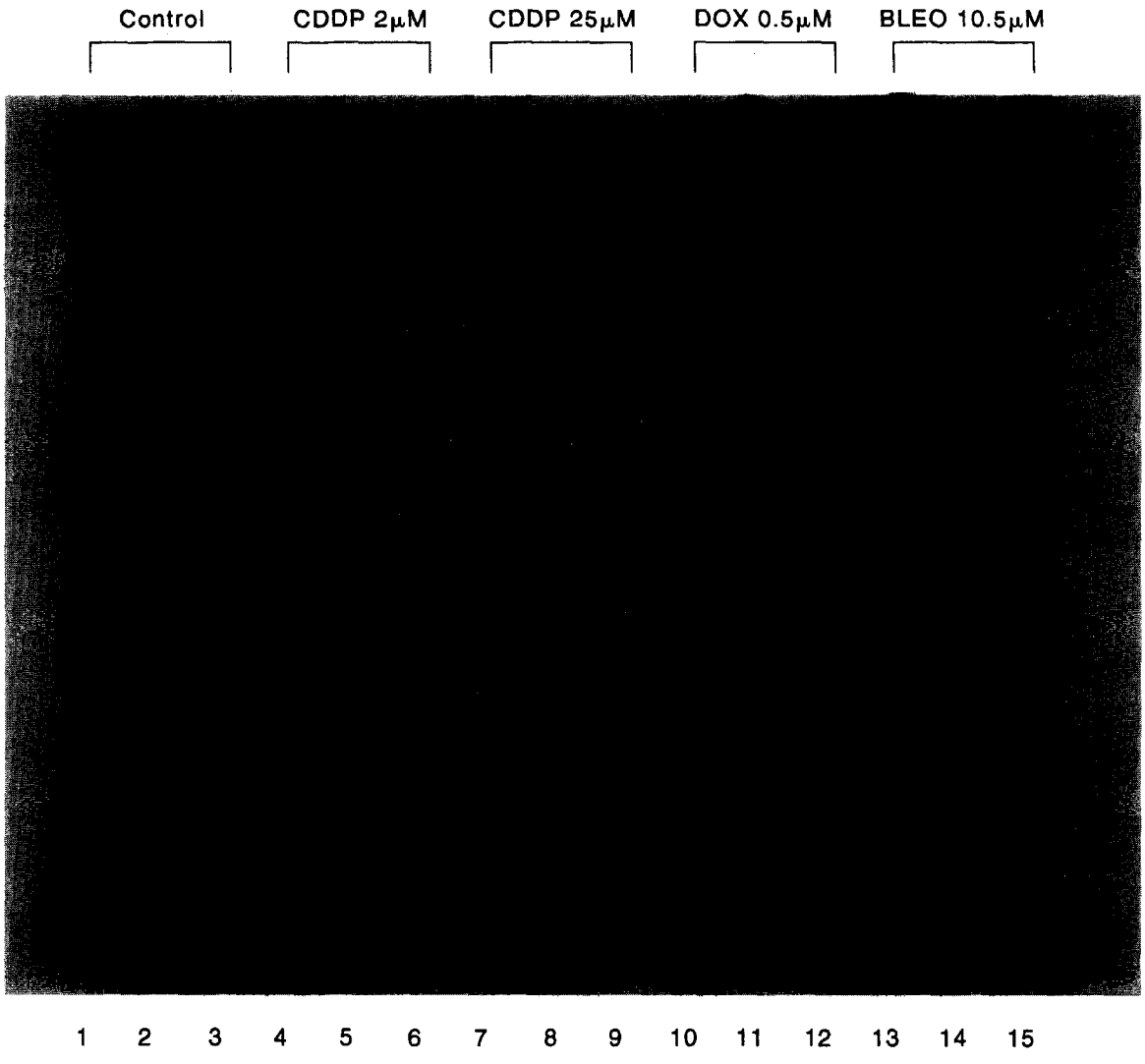

Figure 3. Telomerase activity in the Susa CP testicular cancer cell line after 6 days continuous exposure to drug. Enzyme activity was not detected after cisplatin treatment if concentrations (cisplatin 25 μM) causing total growth inhibition were applied (lane 7 = 0.1 μg total cellular protein, 8 = 0.01 μg protein and 9 = 0.01 μg protein). Less telomerase activity was detected compared to solvent-treated control cells at IC_{50} concentrations (2 μM) of CDDP (lanes 7–9). No enzyme inhibiton was seen at lethal concentrations of DOX (0.5 μM, lanes 10–12) and BLEO (0.5 μM, lanes 13–15) after 6 days exposure to these drugs, concentrations which represent approximately 100 times the IC_{50} for both drugs [16]

affected at IC_{50} concentrations (16 μM for Susa CP) of cisplatin or if telomerase activity was assayed immediately 4 h after drug exposure (Figure 2, 100 μM, lanes 7–9). Dose-dependent telomerase inhibition was also seen after 6 days continuous cisplatin exposure, where concentrations leading to telomerase inhibition were lower (2–25 μM, Figure 3). To test whether free cisplatin could potentially inhibit the PCR reaction, drug was added directly to the reaction tube. Even if 10 μM cisplatin (freshly prepared in water), a concentration which would exceed possible unbound drug concentrations, was added to untreated Susa CP cell extracts, no telomerase inhibition was observed (data not shown). The lack of direct inhibition of telomerase in the PCR reaction, and the need to incubate intact cells in drug-free medium for at least 20 h following exposure to 100 μM cisplatin for 4 h in order to demonstrate telomerase inhibition, is suggestive of a time-dependent process. Furthermore, the time span needed (a total of 24 h) to observe reduction of telomerase activity could be indicative of a target upstream of the enzyme itself, and thus that cisplatin (e.g. G–Pt–G adduct formation) might affect the gene transcription level and, therefore, result in decreased enzyme activity with cellular protein turnover.

To test the possibility that telomerase activity is lost as a result of specific events in intact cells which are associated with the cytotoxic activity of cisplatin, the effect of other cytotoxic agents was investigated. In marked contrast to cisplatin, when DOX, BLEO or MTX were assessed under the same conditions, no effect on telomerase activity was observed (Figures 2 and 3). In addition, cross-linking agents with less intrastrand G-specificity, e.g. MEL and tDDP [22], had little or no inhibitory potential (Figure 4). These data imply that cisplatin is indeed capable of inhibiting telomerase activity in a manner that is related to the G–Pt–G intrastrand cross-linking specificity of the drug.

To delineate the mechanism(s) of this observed inhibition of telomerase activity by cisplatin, we examined a possible direct or indirect interaction of cisplatin with the telomerase

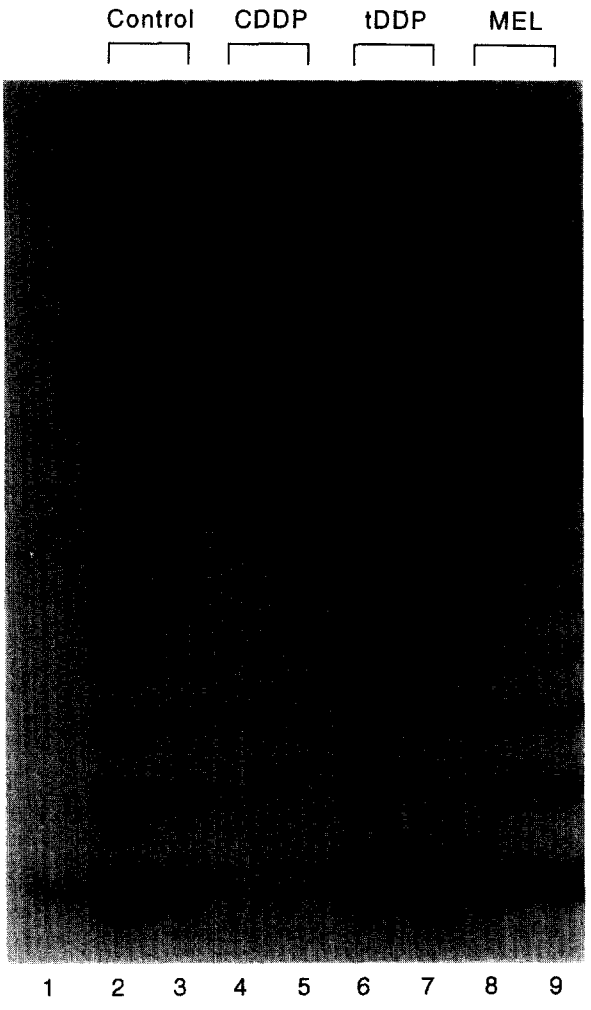

Figure 4. Comparison of the effects of cisplatin, transplatin and melphalan on telomerase activity. Shown is enzyme activity in 0.1 μg (lanes 2, 4, 6, 8) and 0.0 μg (lanes 3, 5, 7, 9) cellular protein after short-term exposure (4 h) to the drugs followed by incubation for 20 h in drug-free medium versus activity in DMA (cisplatin vehicle) treated cells. Cisplatin (CDDP) and melphalan (MEL) were added to Susa CP cells to achieve a final concentration of 100 μM, transplatin (tDDP) to 150 μM. Lane 1 is a lysis buffer blank.

RNA component, which has a GG-rich gene region [13] and is essential for the function of the ribonucleoprotein enzyme. Using hTR component specific primers, cells treated with 100 μM cisplatin, but not vehicle treated control cells or cells exposed to lethal BLEO (5.5 μM) concentrations, showed a significant decrease in hTR cDNA expression (Figure 5). Cisplatin had no effect on the expression of β-actin cDNA, which was used as an internal standard. The detected TRC3 cDNA amplification product from Susa CP control cells was approximately 400 bp and was cleavable at the proposed cutting sites of Hae III, but not as predicted with Eco RI. Eco RI has no cutting site in hTR cDNA, but Hae III did cut at four sites leading to fragment sizes of 102, 97, 75, 74 and 58 bp (data not shown). These results suggest that cisplatin interferes with the transcription of the hTR component, and in agreement with the need to allow cellular proteins to turn over, implies that functional telomerase activity is probably not detectable after 24 h due to a lack of hTR component synthesis in cisplatin-treated Susa CP cells.

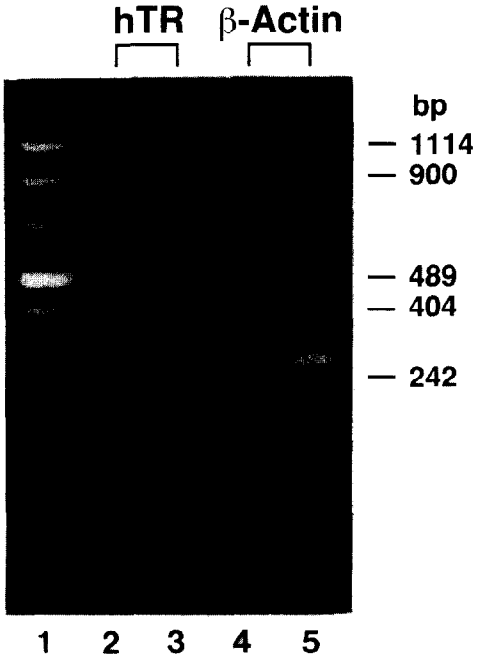

Figure 5. RT-PCR. Telomerase RNA component expression in Susa CP control cells, treated with DMA (lane 2) or 100 μM cisplatin (dissolved in DMA, lane 3), respectively. Depicted are hTR levels in cells 24 h after treatment (4 h exposure to drug/vehicle and 20 h incubation in drug-free medium) in comparison to β-actin expression in the same cell extracts. Susa CP control cells (lanes 2 and 4); cisplatin treated cells (lanes 3 and 5). Lane 1 is a molecular weight marker.

DISCUSSION

The human testicular cancer cell lines Susa and Susa CP have been frequently used as model systems in previous studies in an effort to explain the high success rate in treatment of testicular cancer with cisplatin-based chemotherapy [16, 17, 19, 23–36]. The chemosensitivity of these testicular cancer cells *in vitro* has been attributed to topoisomerase II protein overexpression [25], induction of apoptosis [26], low content of the detoxifying agent glutathione [19] and, moreover, the inability to repair DNA-Pt intrastrand cross-links [23, 24]. Specifically, it has been shown that, following an 18 h post-treatment incubation period, the levels of the major intrastrand G–Pt–G adduct are not reduced in the testicular cell lines examined, which included the Susa lines [23, 24]. These findings were consistent with our hypothesis that telomerase inhibition might be brought about by cisplatin via persistent G–Pt–G adducts in the DNA/RNA regions essential for expression or maintenance of telomerase activity. Since cisplatin's effect on telomerase activity is distinct from other cytotoxic agents as described above, one might propose that inhibition of telomerase activity could, in part, contribute to cisplatin's marked efficacy against germ cell derived tumours and thus, inhibition of telomerase activity might have therapeutic potential.

Detailed investigations to define the precise mechanism underlying the observed inhibition of telomerase activity in human testicular cancer cell lines are ongoing. Several possibilities exist as to how cisplatin might interfere with telomerase function. One possibility is that the telomeric repeat sequence TTAGGG could be cross-linked. Alternatively, interactions of cisplatin with essential sulphydryl groups in the protein part of the enzyme are also possible.

Furthermore, there is evidence from the data presented in Figure 5 that cisplatin might disable transcription of the telomerase-RNA encoding gene region, as the expression of human telomerase RNA component, measured using the hTR specific TRC3 primers described by Feng and associates [13], was significantly diminished. The fact that other DNA damaging agents had no effect on telomerase activity, that BLEO (data not shown) did not reduce hTR cDNA expression, and that the transcription of β-actin cDNA was still functional in cisplatin-treated cells, is indicative of specific DNA lesions exerted by this drug. Further studies will be needed to address whether the observed inhibition of telomerase activity by cisplatin correlates with loss of telomere length in cells and in residual testicular tumour masses following treatment with cisplatin-based regimens.

1. Zakian VA. Telomeres: beginning to understand the end. *Science* 1995, **270**, 1601–1607.
2. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing. *Nature* 1990, **346**, 866–868.
3. Harley CV, Futcher AB, Greider CW. Telomeres shorten during ageing. *Nature* 1990, **345**, 458–460.
4. Kim NW, Piatyszek MA, Prowse KR, *et al.* Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994, **266**, 2011–2015.
5. Rhyu MS. Telomeres, telomerase, and immortality. *J Natl Cancer Inst* 1995, **87**, 884–894.
6. Shay JW, Werbin H, Wright WE. Telomere shortening may contribute to ageing and cancer: a perspective. *Mol Cell Diff* 1994, **2**, 1–21.
7. Bednarek A, Budunova I, Slaga TJ, Aldaz CM. Increased telomerase activity in mouse skin premalignant progression. *Cancer Res* 1995, **55**, 4566–4569.
8. Chadeneau C, Hay K, Hirte HW, Gallinger S, Bacchetti S. Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Res* 1995, **55**, 2533–2536.
9. Morin GB. Is telomerase a universal cancer target? *J Natl Cancer Inst* 1995, **87**, 859–861.
10. Parkinson EK. Do telomerase antagonists represent a novel anti-cancer strategy? *Br J Cancer* 1996, **73**, 1–4.
11. Strahl C, Blackburn EH. The effects of nucleoside analogs on telomerase and telomeres in *tetrahymena*. *Nucleic Acids Res* 1994, **22**, 893–900.
12. Strahl C, Blackburn EH. Effect of reverse transcriptase inhibition on telomere length and telomerase activity in two immortalized human cell lines. *Mol Cell Biol* 1996, **16**, 53–65.
13. Feng J, Funk WD, Wang SS, *et al.* The RNA component of human telomerase. *Science* 1995, **269**, 1236–1241.
14. Norton JC, Piatyszek MA, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase activity by peptide nucleic-acids. *Nature Biotechnol* 1996, **14**, 615–619.
15. Sherman SE, Lippard SJ. Structural aspects of platinum anticancer drug-interactions with DNA. *Chem Rev* 1987, **87**, 1153–1181.
16. Walker CM, Povey S, Parrington JM, Riddle PN, Knuechel R, Masters JRW. Development and characterization of cisplatin-resistant human testicular and bladder tumour cell lines. *Eur J Cancer* 1990, **26**, 742–747.
17. Walker MC, Parris CN, Masters JRW. Differential sensitivities of human testicular and bladder tumor cell lines to chemotherapeutic drugs. *J Natl Cancer Inst* 1987, **79**, 213–216.
18. Einhorn LH, Williams SD. Chemotherapy of disseminated testicular cancer. A random prospective study. *Cancer* 1980, **46**, 1339–1344.
19. Masters JRW, Thomas R, Hall AG, Hogarth L, Matheson EC, Cattan AR, Lohrer H. Sensitivity of testis tumour cells to chemotherapeutic drugs: role of detoxifying pathways. *Eur J Cancer* 1996, **32A**, 1248–1253.
20. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal Biochem* 1976, **72**, 248–254.
21. Piatyszek MA, Kim NW, Weinrich SL, *et al.* Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). *Meth Cell Sci* 1995, **17**, 1–15.
22. Lepre CA, Chassot L, Costello CE, Lippard SJ. Synthesis and characterization of trans-$[Pt(NH_3)_2CL_2]$ adducts of d(CCTCGAGTCTCC·d(GGAGACTCGAGG). *Biochemistry* 1990, **29**, 811–823.
23. Hill BT, Scanlon KJ, Hansson J, *et al.* Deficient repair of cisplatin–DNA adducts identified in human testicular teratoma cell lines established from tumours from untreated patients. *Eur J Cancer* 1994, **30A**, 832–837.
24. Bedford P, Fichtinger-Schepman AMJ, Shellard SA, *et al.* Differential repair of platinum-DNA adducts in human bladder and testicular continuous cell lines. *Cancer Res* 1988, **48**, 3019–3024.
25. Fry AM, Chresta CM, Davies SM, *et al.* Relationship between topoisomerase II level and chemosensitivity in human tumor cell lines. *Cancer Res* 1991, **51**, 6592–6595.
26. Huddart RA, Titley J, Robertson D, Williams GT, Horwich A, Cooper CS. Programmed cell death in response to chemotherapeutic agents in human germ cell tumour lines. *Eur J Cancer* 1995, **31A**, 739–746.
27. Mosmann TJ. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.

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