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Preclinical report

Anti-neoplastic activity of topotecan versus cisplatin, etoposide and paclitaxel in four squamous cell cancer cell lines of the female genital tract using an ATP-Tumor Chemosensitivity Assay

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We evaluated the *in vitro* cytotoxicity of topotecan (TPT), versus cisplatin, etoposide (VP-16) and paclitaxel (PTX) in four squamous cell cancer cell lines of the cervix uteri and vulva. Four established human squamous cancer cell lines from the cervix uteri (A-431, Ca Ski and C-33) and vulva (CAL-39) were used. The cytotoxic effects of the agents were examined using the ATP-Tumor Chemosensitivity Assay (ATP-TCA). In addition to the single agents, the following combinations were tested: TPT+cisplatin, TPT+VP-16 and TPT+PTX. Three cell lines (C-33, Ca Ski and CAL-39) were highly sensitive to TPT, but one cell line (A-431) was less sensitive. Furthermore, the cytotoxic activity of TPT was superior to that of cisplatin in Ca Ski and C-33 cells, but inferior in CAL-39 and A-431. TPT was also more active than VP-16 in CAL-39 and Ca Ski. On the other hand, the cytotoxic activity of TPT was weaker than PTX in C-33, CAL-39 and A-431. TPT increased the cytotoxic activity of cisplatin and VP-16 in C-33, Ca Ski and A-431. However, synergistic features were observed only in A-431 cells. TPT also enhanced the cytotoxic activity of PTX in A-431 and Ca Ski. In CAL-39 and C-33, however, increased cytotoxic activity occurred only at higher drug concentrations, whereas antagonism was observed at lower drug concentrations. In conclusion, our results suggest that TPT has a significant cytotoxic effect on most squamous cell cancer cell lines which may be superior to cisplatin, VP-16 and PTX in some instances. Furthermore, TPT is likely to potentiate the cytotoxic activity of these agents in individual cell lines tested. [© 2000 Lippincott Williams & Wilkins.]

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Introduction

Topotecan (TPT) is a semi-synthethic derivative of camptothecin (CPT), a member of a new class of anti-cancer agents that selectively inhibit the enzyme topoisomerase I. Topoisomerase I has also been demonstrated to be intimately involved in the DNA replication as it relieves the torsional strain introduced ahead of a moving replication fork.[1–4] Inhibition of this enzyme results in lethal damage during the S phase of the cell cycle due to cessation of DNA synthesis and transcription. This finally leads to induction of apoptosis. TPT incorporates a stable basic side-chain at the 9-position of the A-ring of 10-hydroxycamptothecin and retains the *S* configuration at the chiral carbon-20. The basic chain affords water solubility of the compound without requiring hydrolysis of the E-ring lactone that is required for biologic activity.[1,2] The chemical structures of TPT and the parent compound are shown in Figure 1. Preclinically, camptothecin analogs have been shown to exhibit a promising cytotoxic activity in squamous cell carcinomas of both aero-digestive and female genital tracts. Topotecan was compared with cisplatin, etoposide (VP-16) and paclitaxel (PTX), which are known to show cytotoxic activity in cervix cancer and other neoplasms of squamous cell origin.[3,5–8] Four squamous cell carcinoma cell lines of the cervix uteri (Ca Ski, C-33 and A-

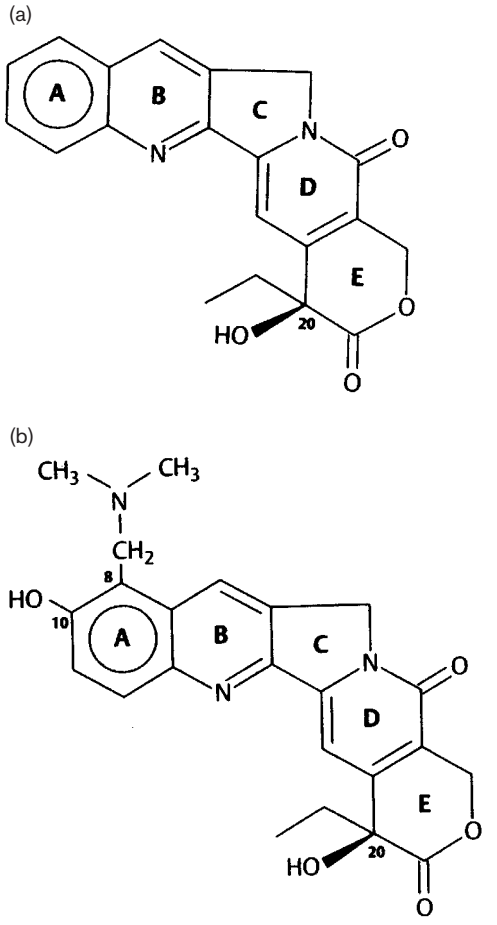

Figure 1. (a) Structure of camptothecin. (b) Structure of topotecan.

431) and vulva (CAL-39) were investigated. Firstly, the influence exerted by TPT as a single agent in the cells was determined, and then in combination with cisplatin, VP-16 and PTX. The drugs were studied, using the ATP-Tumor Chemosensitivity Assay (ATP-TCA). The main purpose of this investigation was to identify (i) whether TPT was an active agent against squamous cell carcinoma of the cervix and vulva, and (ii) whether addition of TPT to established antineoplastic agents would be more effective than the single agents.

Materials and methods

Four established human cell lines selected from squamous cell carcinomas of the cervix (SCCC: Ca Ski, C-33 and A-431) and squamous cell vulvar cancer (SCVC: CAL-39) were studied. Ca Ski and C-33 cells were kindly donated by Dr M Untch (Klinikum Großhadern, Munich, Germany), and A-431 and CAL-39 were obtained from DSMZ (Braunschweig, Germany). All cell lines were maintained in cell culture medium RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10 μg/ml insulin, 100 U/ml penicillin and 100 ng/ml streptomycin at 37°C, in a humidified atmosphere with 5% CO_2 in air. All media and reagents were purchased from Gibco/BRL (Paisley, UK). Cultures were periodically controlled for mycoplasma infection using the 33528 dye (Hoechst, Frankfurt, Germany) and passaged once or twice a week at a 1:2 ratio.

Drugs

For this study, commercial formulations of TPT (Hycamtin®; SmithKline Beecham, Munich, Germany), VP-16 (Vepesid®; Bristol-Myers Squibb), cisplatin (Platinex®; Bristol-Myers Squibb) and PTX (Taxol®; Bristol-Myers Squibb) were used. In addition to single agents, the combinations TPT+cisplatin, TPT+VP-16 and TPT+PTX were assayed. Test drug concentrations (TDCs) were freshly prepared for each experiment and directly performed on the culture plates by serial dilutions of prepared stock solutions. Each agent was tested at six different concentrations with a maximum of 200% TDC and a minimum of 6.25% TDC. *In vitro* referential dosages (100% TDC) corresponding to clinically achievable serum concentrations were TPT (0.46 μg/ml), cisplatin (3.8 μg/ml), VP-16 (48 μg/ml) and PTX (13.6 μg/ml). Each TDC was tested in triplicate. Two controls, one with blank medium (MO) and the other with maximum ATP inhibitor (MI) instead of test drugs, were added to six-well culture plates.

Chemosensitivity assay

In vitro chemosensitivity was determined by the ATP-TCA utilizing a standardized kit technique (TCA 100®; DCS, Innovative Diagnostic Systems, Hamburg, Germany), which was slightly modified in order to test permanent cell lines as previously described in detail.[9–11] Briefly, drugs or drug combinations and cells were pipetted into 96-multiwell micro-plates (Falcon, Becton Dickinson, Heidelberg, Germany) with 10^3 cells/well. Cultures were then incubated for 5 days at 37°C, in a 95% air/5% CO_2 atmosphere with 100% humidity. Each experiment was performed twice.

Assay evaluation

At the end of the incubation time, cellular ATP was extracted and standardized adding 50 μl tumor cell

extraction reagent (TCER) to each well of culture plates. ATP was then measured using a LB 953 luminometer (Berthold, Wildbad, Germany) after automatic addition of 50 μl aliquot of each cell lysate. Counting time was 15 s with a 4 s delay. Luminescence response was expressed as relative light units (RLU=photons/10). Percent tumor growth inhibition (TGI) for each drug and concentration was then determined as $TGI=[1-(RLU_{Test}-RLU_{MI})/(RLU_{MO}-RLU_{MI})\times 100]$. Inhibition data were then graphically converted into individual inhibition curves for each tumor and drug, respectively. ATP standard curves were performed for all assays. To identify a tumor to be completely sensitive or, on the other hand, a particular drug to be active *in vitro*, two criteria were used, i.e. total cell inhibition at high drug concentration (200% TDC) and IC_{50} (drug concentration effecting 50% tumor cell inhibition) below 25% TDC. The combined drug effects were analyzed using the sigmoid model described elsewhere.[12]

Results

Single drug effects

As illustrated in Figure 2(A–D), the cytotoxic actvity of TPT as a single agent was moderate to strong in three of the cell lines tested (C-33, Ca Ski and CAL-39) and weak in A-431. VP-16 as a single agent showed strong cytotoxic activity in C-33 and Ca Ski, and weak cytotoxic activity in CAL-39 and A-431. Cisplatin showed strong cytotoxic activity in CAL-39 and at higher test drug concentrations (between 50 and 200%) in C-33 and Ca Ski as well. The activity of cisplatin was found to be moderate at high concentrations in A-431, and weak at lower TDCs (6.25–25%) in C-33, Ca Ski and A-431. Furthermore, PTX as a single agent showed strong cytotoxic activity in all cell lines tested. Comparison of the cytotoxic activity of the other cytostatics revealed TPT to be superior to cisplatin in Ca Ski and C-33, but inferior in CAL-39 and A-431. TPT was also more effective than VP-16 in CAL-39 and Ca Ski. However, TPT was less active than PTX in all but Ca Ski cells.

Combined drug effects

Results of the combination of TPT with VP-16, cisplatin and PTX are presented in Figure 3(A–D). Compared to the single agents, an increase of cytotoxic activity was observed for TPT+VP-16 in three of the cell lines (C-33, Ca Ski and A-431) and for

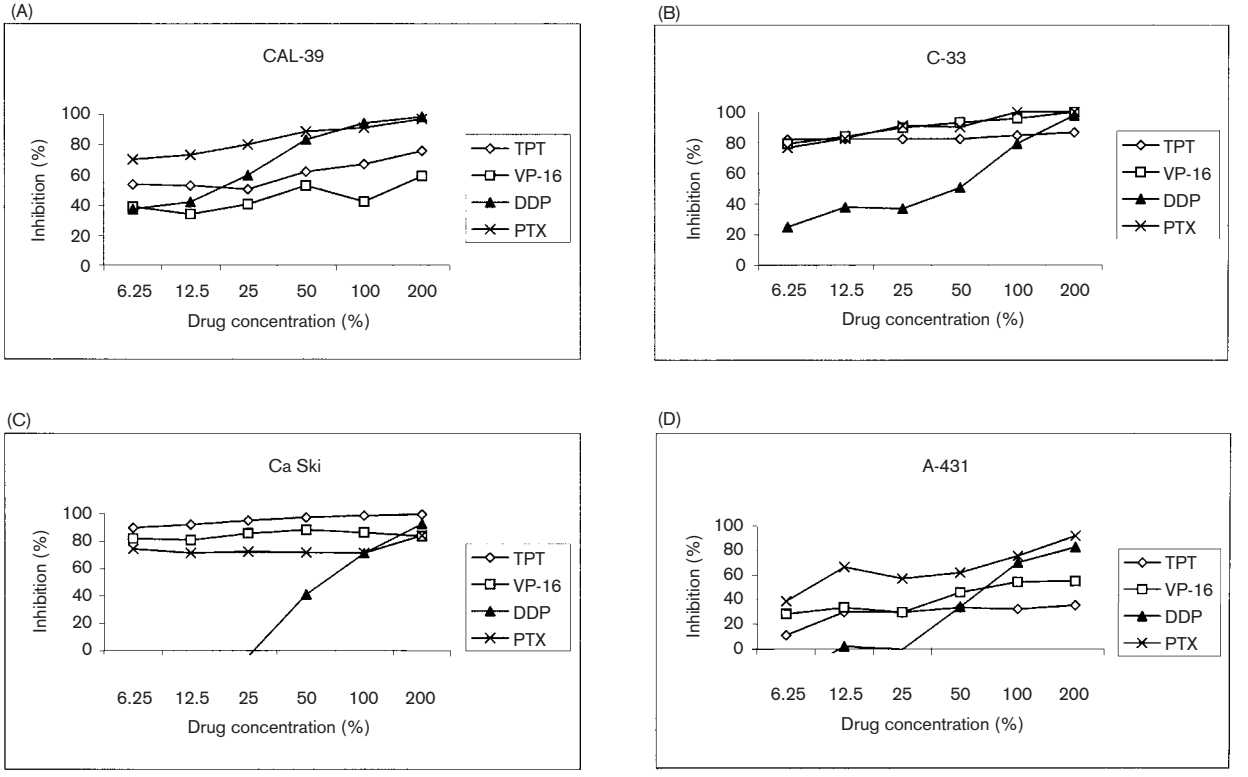

Figure 2. Single drug effects of TPT, cisplatin (DDP), VP-16 and PTX in CAL-39 (A), C-33 (B), Ca Ski (C) and A-431 (D) at variable concentrations.

TPT+cisplatin in two cell lines tested (Ca Ski and A-431), respectively. However, features reminiscent of synergism or major additivity were displayed only in A-431 cells. In CAL-39, these combinations were found to act antagonistically, as was TPT+cisplatin at lower concentrations in C-33. Furthermore, in A-431 major additivity or synergistic effects were observed with the combination TPT+PTX. However, in two of the cell lines (CAL-39 and C-33), antagonistic effects were observed at lower test drug concentrations (between 6.25 and 12.5% TDC).

Discussion

ATP bioluminescence assay is considered to be a promising means for the assessment of chemosensitivity in both tumor cell lines and native tumor specimens.[12–15] This method is proposed for routine pretherapeutic drug screening in clinical oncology.[9,12–16] Additionally, ATP chemosensitivity testing might be valuable for studying the activity of possible anticancer agents *in vitro*.[14] When analyzing the overall *in vitro* response rates, TPT showed a strong antineoplastic activity in three of the cell lines tested (C-33, Ca Ski and CAL-39). However, the anti-neoplastic activity of TPT was weak in A-431 which exhibited the highest overall drug resistance among all cell lines studied.

When compared to cisplatin, VP-16 and PTX, TPT was stronger than VP-16 in the cell lines CAL-39 and Ca Ski. However, TPT was weaker than PTX in C-33, CAL-39 and A-431. The cytotoxic activity of TPT was higher than cisplatin in Ca Ski and C-33, but weaker in CAL-39 and A-431. These results are interesting, because cisplatin and its combinations are currently considered as the therapy of choice in advanced squamous cell carcinoma of the cervix.[5,6] In addition, the degree of efficacy of TPT in squamous cell cancer cell lines according to our results are comparable to other anti-neoplastic agents such as VP-16 and PTX. Generally, these results are consistent with other experimental experience.[11,17–19] The combination of TPT with cisplatin and VP-16 produced an increase of tumor cell inhibition in the two cell lines (Ca Ski and A-431) and the three cell lines (C-33, Ca Ski and A-431), respectively, whilst one cell line (CAL-39) showed a decrease. However, features of synergism or at least major additivity were only found when both single agents were relatively inactive, as was observed in the

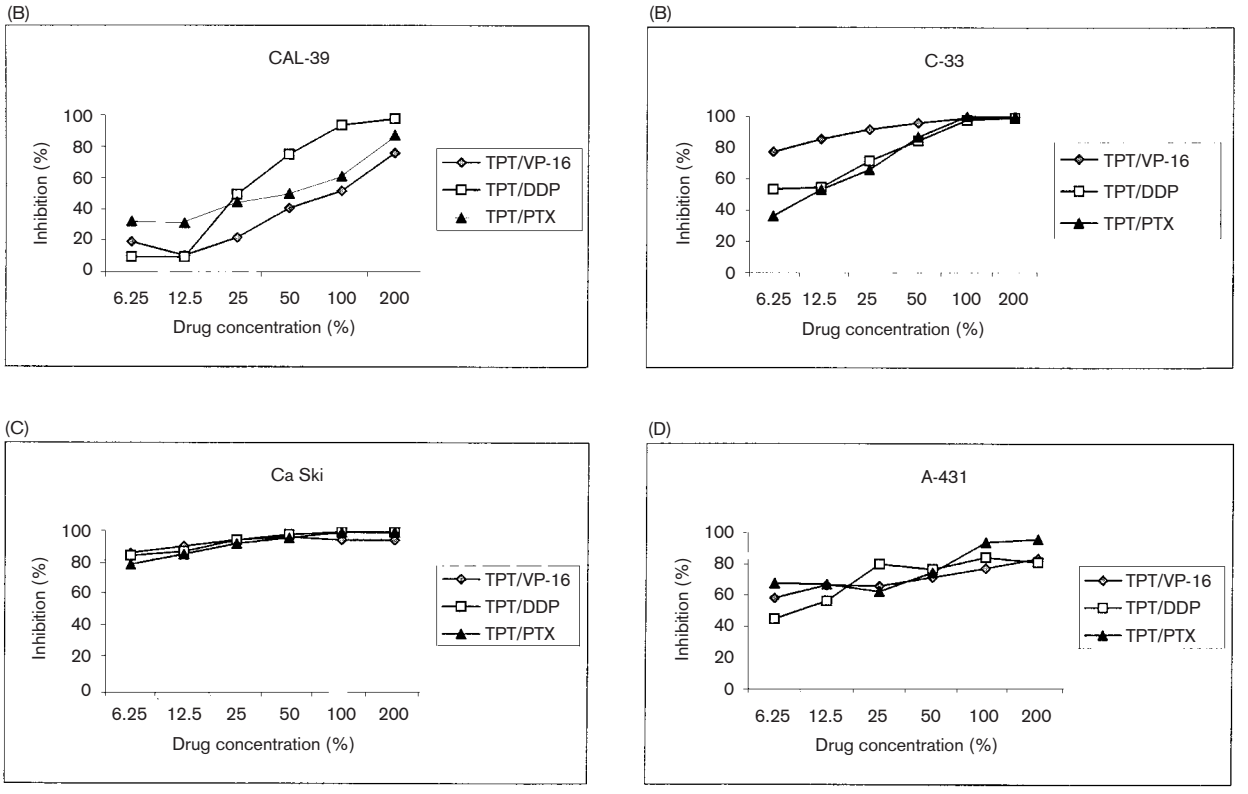

Figure 3. Combined drug effects of TPT with cisplatin (DDP), VP-16 and PTX in CAL-39 (A), C-33 (B), Ca Ski (C) and A-431 (D) variable concentrations.

cell line A-431. Furthermore, positive and negative combination effects of TPT and cisplatin could be observed in one individual cell line tested (C-33) confirming the dose dependency of these effects at least in some gynecological squamous cell cancers.

The combination effects of TPT and VP-16 may be explained by a synergistic inhibitory action of both drugs on DNA repair. Topotecan is likely to potentiate the efficacy of topoisomerase II inhibitors like VP-16, since topoisomerase II levels are known to be upregulated when topoisomerase I is inhibited.[18] The higher cellular content of the biochemical target results in a higher sensitivity of the tumor to a subsequent treatment with etoposide or other topoisomerase II poisons.[8] These effects were found to be optional, when both drugs did produce little or no significant activity *per se*, whereas only minor combination effects were observed in cell lines exhibiting high single-agent sensitivity potentially related to a low increase in topoisomerase content. Another explanation for these individually different combination activity observed may be the fact that sequences of single agents may lead to more intensive topoisomerase inhibition rather than simultaneous application as we used in our experiments.

Additive effects of TPT with cisplatin or VP-16 in mice bearing implanted lewis lung carcinoma have been described previously,[19] confirming our results. However, the combination of TPT with PTX produced different results. Major additive or synergistic effects were observed in only one cell line (A-431), whereas both drugs showed minor combination effects in Ca Ski. In two cell lines (CAL-39 and C-33) both drugs produced a remarkable antagonism when they were given simultaneously. The effect was most pronounced at low drug concentrations.

Our results are partly consistent with and partly contradictory to the results obtained by Debernards *et al*.[20] In this study, it was suggested that PTX may interfere with the activity of camptothecin, a semisynthetic analog of TPT. This observation is not likely to be related to functional or structural similarities since both drugs differ in their cellular targets or major aspects of chemical structure (see Figure 1). The fact that the antagonism between TPT and PTX occurred mostly at lower TDCs suggests that the combined effects of TPT and PTX may be dose dependent, and that these drugs may not necessarily interfere with one another. Therefore increasing the dose as witnessed at higher TDCs in our study, may overcome overt antagonism, as may be sequential rather than simultaneous application.

To our knowledge, this is the first report on the activity of TPT alone, and in combination with VP-16, cisplatin and PTX in squamous cell carcinoma cell lines of cervical and vulvar origin using ATP-TCA. Dependent on the cell line tested, TPT showed significant anti-neoplastic activity, and was able to increase the cytotoxicity of cisplatin, VP-16 and PTX in some instances. These combinations could be of particular interest in the treatment of squamous cell cancer of the cervix and vulva, if these results are confirmed by further studies using native squamous cell carcinomas and drug sequences currently ongoing in our laboratory. The unusually broad spectrum of activity of TPT has been demonstrated to extend to sublines of chemosensitivity tumors with acquired multidrug resistance.[11] The unique mechanism of activity and seemingly lack of cross-resistance to other classes of anticancer drugs argue in favor of incorporating TPT into treatment strategies for both primary and relapsed squamous cell carcinomas of the female genital tract and other origin.

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References

1. Capranico G, Zunino F. DNA-topoisomerase-trapping antitumor drugs. *Eur J Cancer* 1992; **28A**: 2055–60.
2. Del Bino G, Lassota R, Darzynkiewicz Z. The S-phase cytotoxicity of camptothecin. *Exp Cell Res* 1991; **193**: 27–35.
3. Horwitz SB, Horwitz MS. Effects of camptothecin on the breakage and repair of DNA during the cell cycle. *Cancer Res* 1973; **33**: 2824–36.
4. Hsiang Y, Hertzberg R, Hecht S, *et al*. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; **260**: 14873–8.
5. Alberts DS, Mason-Liddil M. Role of cisplatin in the management of advanced squamous cell carcinoma of the cervix: a gynecologic oncology group study. *Gynecol Oncol* 1989; **34**: 357–9.
6. Hoskins WI, Perez C, Young RC. Gynecologic tumors. In: De Vita Jr VT, Hellmann S, Rosenberg SA, eds. *Cancer: principles and practice of oncology*, 3rd edn. Philadelphia, PA: Lippincott 1989: 1099–161.
7. Sevin BU, Perras JP, Averette HEA, Donato DM, Penalver M. Chemosensitivity testing in ovarian cancer 1993; **71**(suppl 4): 1613–20.
8. Wani MC, Taylor HL, Wall ME, *et al*. Plant antitumor agents VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 1971; **93**: 2325–7.

9. Kurbacher CM, Bruckner HW, Andreotti PE, Kurbacher IA, Sass G, Krebs D. *In vitro* activity of titanocenedichloride versus cisplatin for ovarian carcinoma cell lines evaluated by a microtiter plate ATP bioluminescence assay. *Anti-Cancer drugs* 1995; **6**: 697–704.
10. McCabe FL, *et al*. Chemotherapeutic effectiveness of topotecan against murine colon carcinomas in mice. *SK & F Report* 1988; 008 CM.
11. McCabe FL, *et al*. Chemotherapeutic effectiveness of topotecan against multidrug resistant sublines of p388 Leukemia in B6D2F1 mice. *SK & F Report* 1988; 010 CM.
12. Poch G, Vychodil-Kahr S, Petru S. Sigmoid model versus median-effect analysis for obtaining dose–response curves for *in vitro* chemosensitivity testing. *Int J Clin Pharmacol Ther* 1999; **37**: 189–92.
13. Ahman F, Garewall A, Schiffmann R, Celniker A, Rodney S. Intracellular adenosine triphosphate as a measure of human cell viability and drug modulated growth. *In vitro Cell Dev Biol* 1987; **23**: 474–80.
14. Andreotti PE, Ihornwaite JT, Morse IS. ATP tumor chemosensitivity assay. In: Stanley PE, Kricka LJ, eds. *Bioluminescence and chemiluminescence: current status*. Chichester: John Wiley 1991: 417–20.
15. Sevin BU, Peng Z, Perras J, Ganjei P, Penalver G, Averette H. Application of an ATP tumor chemosensitivity testing. *Gynecol Oncol* 1988; **31**: 191–204.
16. Kurbacher CM, Nagel W, Mallmann P, *et al*. *In vitro* activity of titantocenedichloride in human renal cell carcinoma compared to conventional antiphlogistic agents. *Anticancer Res* 1994; **14**: 1529–34.
17. Mattern RR, Mong SM, Bartus HF, *et al*. Relationship between intracellular effects of Camptothecin and the inhibition of topoisomerase I in cultured L 1210 cells. *Cancer Res* 1987; **47**: 1793–8.
18. McCabe FL, *et al*. Chemotherapeutic effect of topotecan against breast adenocarcinoma 16/C in female C3H/Hemice. *SK & F Report* 1988; 003 CM.
19. McCabe FL, Fancelte LF, Johnson RK. Chemotherapeutic effects of topotecan against lewis lung carcinoma in female B16D2 F1 mice. *SK & F Report* 1988; 004 CM.
20. Derbernards D, Cimoli G, Parodi S, Russo P. Interactions between taxol and campthothecin. *Anti-Cancer Drugs* 1996; **7**: 531–4.
21. Liu DF. DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 1989; **58**: 351–74.
22. White CM, Zborowski E, Gordon NH, Mackay W, Berger NA. Topotecan increases topoisomerase II alpha levels and sensitivity to treatment with etoposide in schedule-dependent process second-line therapy. *Cancer Res* 1997; **11**: 21–8.
23. Zhang H, Drapa P, Liu LF. A model for tumor cell poisons. *Cancer Cells* 1990; **2**: 23–7.

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