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Clinical report

Interactions between taxol and camptothecin

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Taxol is an antitumor drug which, as its mechanism of action, promotes microtubule assembly *in vitro*. Camptothecin (CPT) is an anticancer agent with the peculiar mechanism of poisoning eukaryotic DNA topoisomerase I. Both drugs are in clinical trials and their chemotherapeutic efficacy seems promising in refractory human ovarian cancer. We studied the molecular and cellular pharmacology of the two drugs when administered simultaneously toward human ovarian cancer cell line A2780. Taxol inhibits CPT-induced single-strand breaks as well as CPT-induced cytotoxicity. Taken together, our experiments indicate that the two drugs might interact with the same class of nuclear enzyme, i.e. DNA topoisomerase I.

Key words: Campthotecin, mechanism of action, taxol, topoisomerase I.

Introduction

Taxol, a diterpenoid originally isolated from the stem bark of the western yew, *Taxus brevifolia*,[1] exerts its antineoplastic activity against different murine cancer cells (B16 melanoma, L1210 and P388 mouse leukemia cells) as well as human (MX-1 mammary tumor, CX-1 colon and LX-1 lung tumor xenografts),[2,3] by inhibiting mitosis through enhancement of the polymerization of tubulin and consequent stabilization of microtubules.[4] Taxol has been entered in phase I and II trials. Promising results are obtained in refractory human ovarian cancer.[5,6]

Camptothecin (CPT) is also one of the most promising agents in the treatment of refractory human ovarian cancer;[7,8] this drug is well known to react with DNA topoisomerase I[9] with a unique mechanism of action. CPT is characterized by its ability to form DNA–topoisomerase I cleavable complexes well detected as protein-associated DNA strand-breaks in mammalian cells by the alkaline elution technique, under deproteinizing conditions,[10] which are equivalent to the enzyme–DNA complexes observed in purified systems.[10] The two drugs appear to have distinctly different mechanisms of action. These observations, however, do not necessarily predict a possible synergistic action of the two drugs when administered simultaneously. To determine this point accurately we have exposed A2780 human ovarian cancer cell line to different concentrations of taxol in combination with CPT for 1 h and assessed growth inhibition and cleavable complex formation. Surprisingly, taxol inhibits CPT-induced single-strand breaks as well as CPT cytotoxicity. Taken together, our experiments indicate that the two drugs might interact with the same class of nuclear enzyme, i.e. DNA topoisomerase I.

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Materials and methods

Reagents and cells

A2780 human ovarian cancer cell line was utilized for this study. Cells were maintained as monolayer cultures in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and 1% gentamicin. The doubling time is 24 ± 1 h and the modal chromosome number is 46. Taxol was a generous gift of Bristol-Myers Squibb (Princeton, NJ) and purchased from Calbiochem (San Diego, CA). CPT was obtained from Sigma (St Louis, MO). Both drugs were dissolved in 100% dimethyl-sulfoxide at a concentration of 10 mM and stored frozen. Drugs were diluted in RPMI 1640/10% FCS and immediately used.

Cytotoxicity assay

Drug-induced cytotoxicity was determined by the inhibition of colony formation assay (ICFA) (drug treatment for 1 h). Cells (500/2.5 ml) were plated in six-well Nunc dishes and allowed to attach for 18 h at 37°C. Cells were treated with drug for 1 h, then the drug was removed by washing (two times) and cells were allowed to grow for 12–14 days. Colonies were stained with 0.5% methylene blue in 50% methanol and counted. The plating efficiency of the untreated cells was 32.6% and used to normalize for drug-induced cytotoxicity. The IC_{50} value represents the 50% inhibitory concentration and was calculated by linear interpolation of the values immediately higher and lower than 50% inhibition.

Determinations of DNA single-strand breaks (SSBs)

For the determination of drug-induced DNA breaks in A2780 cells, the alkaline elution technique, under deproteinized conditions, was used as described by Cimoli *et al.*[11] Briefly, cells were seeded (2.5×10^6 cells/150 cm^2) and labeled with [^{3}H]thymidine (0.2 μCi/ml) for 24 h. The medium was then changed and the cells were grown in fresh medium for an additional 24 h to allow the incorporation of the label into high molecular weight DNA. The cells were then exposed to different concentrations of drug for 1 h at 37°C. Cells were scraped in Hanks' balanced salt solution and alkaline elution, under deproteinizing conditions, was performed as described previously.[11] All the results obtained with the alkaline elution assay were expressed as percentage of fraction of DNA retained on the filter after 15 h of elution at PH 12.1

Results and discussion

Initially the activity of CPT or taxol alone was evaluated in A2780 cells using the growth inhibition assay (Figure 1A). Cells were very sensitive to CPT but moderately resistent to taxol; the IC_{50} values (i.e. the drug concentrations required to inhibit cell growth by 50%) were 0.33 and greater than 100 μM), respectively. In a second set of experiments cells were treated simultaneously with 1 or 10 μM CPT in the presence of different concentrations of taxol (from 0.01 to 100 μM) (Figure 1B). Taxol was able to inhibit the CPT cytotoxic effects and this ability was taxol dose dependent.

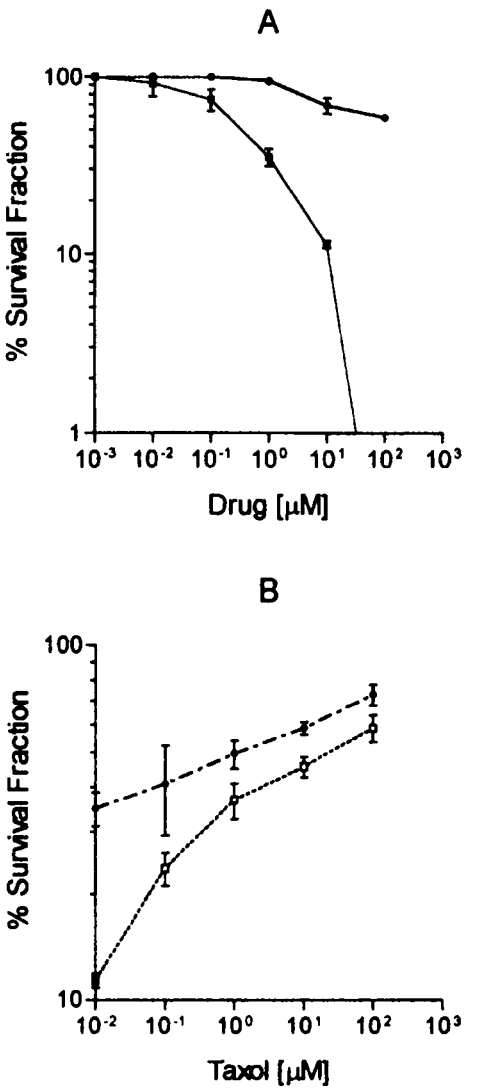

Figure 1. (A) CPT alone (■), taxol alone (●). (B). Cytotoxicity of CPT 10 μM + different concentrations of taxol (●) or CPT 10 μM + different concentrations of taxol (□) against A2780 cells. Cells were incubated for 1 h at 37°C. Cytotoxicity was measured by colony-forming assays. Symbols indicate means and bars indicate standard error of at least three independent experiments.

CPT alone (from 0.1 to 10 μM) induces a dose-dependent high level of DNA SSB (Figure 2A), evaluated as percentage (%) of DNA fraction eluted by the filter. Taxol does not induce or induces few SSBs at very high concentrations (500 μM). These breaks were detectable only under deproteinizing conditions (with PVC filters, high ionic strength lysis solution and no proteinase K, the percent of DNA retained on filter was 93.8 ± 0.4). In the combination experiments, taxol was added to the cell

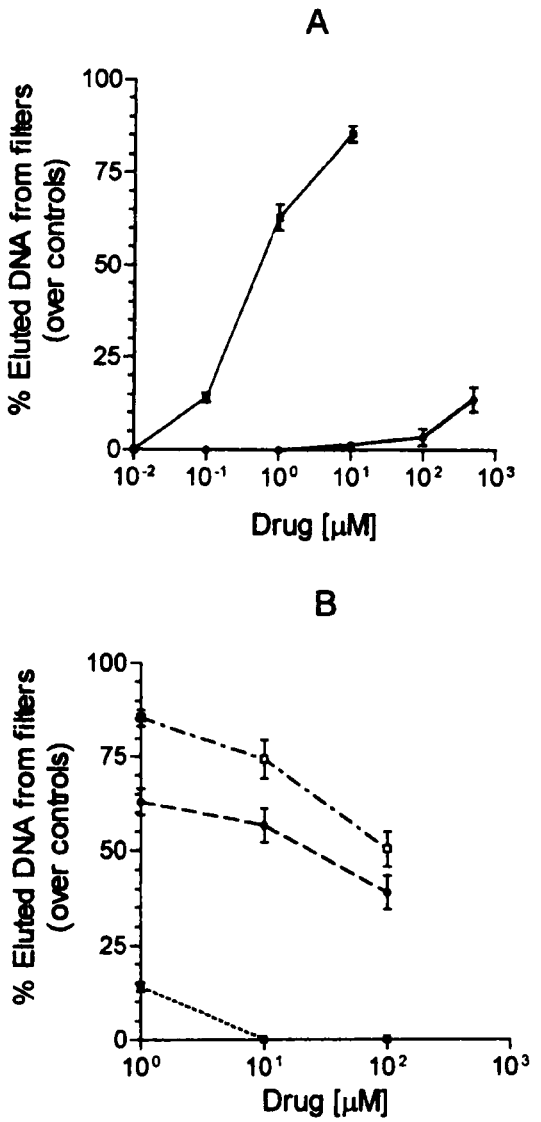

Figure 2. Drug-induced DNA fragmentation in A2780 cells. Drug-induced DNA fragmentation was measured by the alkaline elution technique, under deproteinizing conditions, after 1 h treatments, as previously reported.[11] Data are expressed as percentage of fraction of DNA eluted by the filter after 15 h of elution at pH 12.1. Symbols indicate means and bars indicate standard error of at least three independent experiments. (A) CPT alone (■), taxol alone (●). (B) CPT 0.1 μM (■), 1 μM (●) or 10 μM (□) + different concentrations of taxol.

medium 5 min after the CPT treatment and then the treatment continued for an additional 55 min. Taxol at different concentrations (from 1 to 100 μM) was able to completely inhibit the breaks induced by 0.1 μM CPT and mostly the breaks induced by 1 or 10 μM CPT (Figure 2B). It is important to note that taxol seems to act at the same range concentrations (1–10 μM) required to stabilize microtubule formation.

It is well-known that all the DNA strand breaks, that could be evaluated by the alkaline elution methodology under deproteinizing conditions, produced by CPT are protein linked, and that SSB and DPC frequencies are of a 1 : 1 ratio. These results provide some evidence for the involvement of topoisomerase I-mediated DNA cleavable complex formation as the unique mechanism for the induction of DNA breaks by CPT in mammalian cells.[10,11] Recently, Tanizawa *et al.*[10] also reported a good relationship between the extent of SSB formation and cell killing induced by CPT and, in conclusion, they considered the measurement of protein-linked DNA SSBs as a good indicator of topoisomerase I inhibition and drug activity. Our results clearly indicate that taxol inhibits CPT cytotoxicity as well as CPT-induced SSBs in A2780 human ovarian cancer cell line. Working in isolated nuclei, which avoids the problem of cellular drug uptake, we obtained the same results, i.e. inhibition of CPT-induced SSBs by taxol (data not shown).

Conclusion

Taken together these data suggest that taxol might interact with the same target of CPT, i.e. DNA topoisomerase I. We are currently investigating this possibility.

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