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Paclitaxel sensitizes multidrug resistant cells to radiation

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The unique action of paclitaxel, to stabilize microtubules and block cells at the radiosensitive G_2M phase of the cell cycle, suggests it may sensitize tumors to radiotherapy. We have investigated the potential of this interaction to overcome multidrug resistance *in vitro* using the HL60 cell line and its P-glycoprotein expressing, multidrug resistant H/E8 subline. HL60 cells showed a modest 1.4-fold ($p < 0.01$) increase in sensitivity to 2 Gy radiation given 24 h after a 1 h treatment with paclitaxel. The H/E8 subline, which has increased radiation resistance and expresses an extended multidrug resistance phenotype, showed significant sensitization to radiation (up to 2.3-fold sensitization; $p < 0.01$) even with doses of paclitaxel which had no effect on cell viability or were associated with any G_2/M block in the cell cycle. In the presence of verapamil, an inhibitor of P-glycoprotein mediated efflux, drug resistant cells could be sensitized to 2 Gy radiation by similar paclitaxel doses as the parental cell (≥ 30 nM; $p < 0.01$). These results indicate a therapeutic advantage may be possible in the treatment of resistant tumors by the combined use of paclitaxel with radiation.

Key words: Multidrug resistance, paclitaxel (taxol), P-glycoprotein, radiation.

Introduction

Development of drug resistance is a major problem in the treatment of cancer. Both at initial diagnosis or at relapse, patients present with disease that is refractory to treatment. The most common mechanism associated with the development of drug resistance is the expression of P-glycoprotein. This is a membrane protein which confers drug resistance by acting as a drug efflux pump and so decreases the intracellular drug accumulation.[1] While expression of P-glycoprotein does not account for all refractory tumors, expression of this well characterized protein is generally associated with poor disease outcome.[2] More recently, characterization of a multidrug resistance-associated protein (MRP) has provided an explanation for the resistance demonstrated by many of the non-P-glycoprotein multidrug resistant cell lines and tumors.[3]

An aim of cancer research therefore has been to develop new drugs or treatment regimens which can either circumvent or prevent the development of drug resistance. One of the most successful new drugs in the treatment of cancer has been paclitaxel. Paclitaxel has been shown to be effective in drug refractory ovarian carcinoma, in previously treated patients with metastatic breast cancer and in advanced small cell lung cancer.[4–6] There is an increasing interest in the use of paclitaxel in the treatment of solid tumors due to its unique mechanism of action. Paclitaxel enhances microtubular assembly and prevents its depolymerization, thereby blocking cells in the G_2/M phase of the cell cycle.[7] Since the G_2/M phase of the cell cycle is known to be the most radiation sensitive phase, paclitaxel is being used in combination with radiation in clinical trials for locally advanced breast cancer[5] and advanced non-small cell lung cancer.[8]

In vitro studies into the effect of paclitaxel on cells treated with radiation have shown that paclitaxel is able to induce radiation sensitization,[9–15] although the involvement of the G_2/M block in this enhancement effect is in doubt.[13,16] One potential problem in the use of paclitaxel as a radiosensitizer is that this natural product drug is effluxed by the multidrug transporter P-glycoprotein.[7] We have recently described the development of an extended multidrug resistant HL60 subline (H/E8) which expresses P-glycoprotein and is also resistant to the DNA binding drugs cisplatin and chlorambucil, drugs not associated with the multidrug resistance phenotype.[17] Further, these cells have increased radiation resistance, and so more closely reflect the type of extended drug and radiation resistance encountered in the treatment of cancer. We have

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therefore examined the effect of paclitaxel and radiation in these extended multidrug resistant HL60 cells.

Materials and Methods

Cell Lines

The HL60 human promyelocytic cell line[18] was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 medium (ICN-Flow, Sydney, Australia) supplemented with 10% fetal calf serum (Cytosystems, Sydney, Australia), 20 mM HEPES (Cytosystems) and $NaHCO_3$ (0.85 g/l) at 37°C in a humidified atmosphere with 5% CO_2. The drug resistant H/E8 subline was developed by intermittent treatment with epirubicin as previously described.[17] All cultures were free of mycoplasma.

Paclitaxel sensitivity

Sensitivity to paclitaxel was determined using the MTT cell viability assay as previously described.[19] Briefly, exponentially growing cells (10^4) were plated in triplicate in 200 μl media containing 2-fold drug dilutions. After 4 days incubation, cell survival was determined using MTT. Paclitaxel was dissolved in dimethylsulfoxide to give a stock solution of 1.0 mg/ml. Further dilutions were made in culture medium and the concentration of vehicle used in all experiments had no effect on cell viability. Determinations were in triplicate and all assays were repeated at least twice. The 50% inhibitory concentration (IC_{50}) was determined as the drug concentration which resulted in a 50% reduction of cell viability. Statistical significance was evaluated by the Student's *t*-test.

Radiation sensitivity

Radiation sensitivity was determined in a similar way as described above for drug sensitivity and the assay was optimized to ensure continuous growth of control wells as described by Carmichael *et al.*[20] The 96-well plates (Cytosystems) containing 10^4 cells/well were treated with a gradient of γ-radiation at a maximum dose rate of 1.2 Gy/min using a ^{60}Co source housed in a Theratron 780 radiotherapy treatment machine and fitted with a linear wedge shaped lead filter placed across the field of irradiation. A 5 min exposure gave an average cell dose range of 1.6 Gy in column 2 of the microtiter plate to 6 Gy in column 11 of the plate. Each well in a column received the same dose, and wells in the outside rows and columns of the plate were not used. The radiation dose received by each column was determined using thermofluorescent densitometry of 2 × 2 mm lithium fluoride chips exposed under experimental conditions. Cell viability was determined using MTT after 5 days incubation at 37°C. The LD_{50} was calculated as the dose required to cause a 50% reduction in the cell viability.

Combined paclitaxel and radiation studies

Cells were seeded into 10 ml of culture medium (10^5/ml) and incubated for 48 h to ensure exponential growth. Paclitaxel was added for 1 h after which cells were centrifuged (800 *g* for 5 min) and resuspended in 10 ml of fresh culture medium prior to incubation for a further 24 h. After this period, cells were centrifuged (800 *g* for 5 min), resuspended in culture medium and plated in 96-well plates for determination of radiation sensitivity. Aliquots (10^6 cells) were removed for cell cycle analysis. Cell survival was calculated for each concentration of paclitaxel using the relevant non-irradiated control. Sensitization was determined as the cell viability for control untreated cells divided by the cell viability of the treated cells.

Clonogenic assay

Cells were suspended in 1 ml 3% agar in RPMI 1640 containing fetal calf serum (25%), 20 mM HEPES, $NaHCO_3$ (0.85 g/l), bovine serum albumin (1%) and mercaptoethanol (1%), and plated in duplicate on a 2 ml feeder layer of 0.5% agar in the same medium in a 6-well plate (Cytosystems). Plates were incubated for 12 days at 37°C in a humidified atmosphere containing 5% CO_2. Clones containing more than 30 cells were counted as viable. The plating efficiency under these conditions for both cell lines was 15–20%. The D_0 (-1/slope) was calculated by linear regression and the relative resistance was calculated as the D_0 for the resistant cell line divided by the D_0 for the sensitive cell line.

Cell cycle analysis

Cells (10^6) were washed and resuspended in 500 μl PBS containing propidium iodide (50 μg/ml) and 0.02% Nonidet P-40. Cells were incubated on ice for 15 min and analyzed on a Becton Dickinson FACScan flow cytometer using CellFit data analysis. All cell cycle analyses were in duplicate and each experiment was repeated at least twice.

Drugs and Reagents

MTT, paclitaxel, dimethysupfoxide, mercaptoethanol, propidium iodide, Nonidet P-40 and verapamil were purchased from Sigma (St Louis, MO). Noble agar was from Difco (Detroit, Mi) and bovine serum albumin from Boehringer Mannheim (Mannheim, Germany). All other chemicals were AR grade.

Results

The effect of paclitaxel on HL60 cells or the multidrug resistant H/E8 subline was dependent on both dose and time of drug exposure. For the HL60 cells in a 4 day MTT cytotoxicity assay, continuous exposure to the drug resulted in an IC_{50} of 2.7 ± 0.2 nM ($n=3$). However, removal of paclitaxel after 1 h followed by 4 days incubation in a drug-free media gave an IC_{50} of 59 ± 2.0 nM ($n=3$). Similarly for the H/E8 subline, a 4 day continuous exposure MTT assay gave an IC_{50} of 20.5 ± 1.4 nM ($n=3$), while for a 1 h treatment with paclitaxel the IC_{50} was 3085 ± 250 nM ($n=3$). Therefore the relative resistance of the H/E8 subline is even higher after a 1 h exposure to paclitaxel (52-fold) compared with the relative resistance determined in a 4 day exposure cytotoxicity assay (8-fold).

The H/E8 subline has previously been reported to be radiation resistant compared with the parental HL60 cells.[17] Using the MTT assay, 2.8 ± 0.4 Gy ($n=8$) caused 50% cell death in the HL60 cells, while a 4.6 ± 0.5 Gy ($n=8$) dose was required for a similar cell death in the H/E8 subline (1.64-fold resistance; $p<0.001$). Figure 1 demonstrates the radiation resistance of the H/E8 subline relative to the HL60 cells by both MTT colorimetric assay and clonogenic assay. By clonogenic assay the relative resistance calculated from the D_0 values was 1.75. As the optimized MTT assay reflects cell survival, it was used to assess cell viability after combined treatments of paclitaxel and radiation.

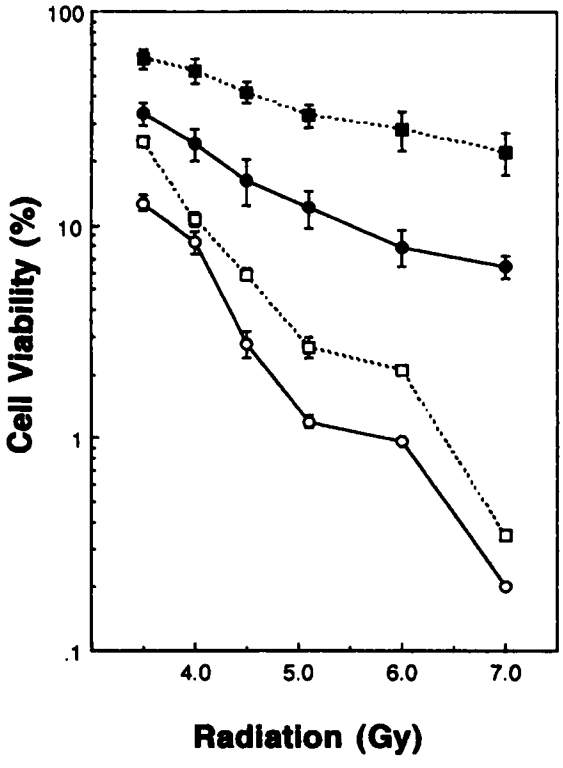

Figure 1. Radiation resistance. HL60 cells (circles) and the H/E8 drug resistant subline (squares) were treated with radiation and the cell viability assessed by MTT cell viability assay (solid symbols) and clonogenic assay (open symbols). The standard deviation is shown.

Exponentially growing cells were treated with a 2–200 nM dose range of paclitaxel for 1 h for the HL60 cells and a 200–4000 nM dose range for the H/E8 subline. After 24 h incubation, cells were exposed to radiation doses ranging from 1.6 to 6 Gy. Figure 2 shows the effect of pretreatment of HL60 cells with 20 or 50 nM paclitaxel compared with the untreated cells. Cell viability is presented both as the absorbance values from the MTT assay (Figure 2a) and relative to the unirradiated cells treated with the same dose of paclitaxel (Figure 2b). For HL60 cells treated with 20 nM paclitaxel, a concentration well below the IC_{50} dose of 59 ± 2.0 nM, paclitaxel had little effect on cell viability after radiation. At doses of paclitaxel which alone resulted in approximately 50% cell viability (50 nM; Figure 2a, zero radiation), there was a slight sensitization to the radiation exposure (1.4-fold; $n=3$), with 2 Gy causing a significant difference between untreated cells and cells treated with paclitaxel ($p<0.01$).

For the H/E8 subline, treatment with doses of paclitaxel which alone caused 50% cell death (3

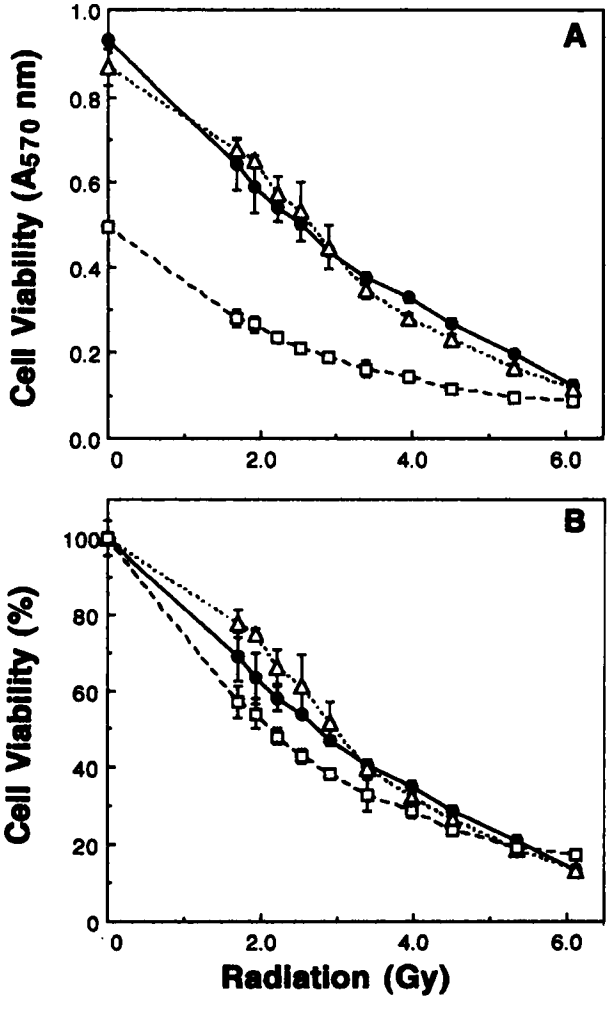

Figure 2. Radiation survival curves of HL60 cells pretreated with paclitaxel. HL60 cells were treated for 1 h with 20 nM (△) or 50 nM (□) paclitaxel. After 24 h, treated and control untreated cells (●) were exposed to radiation. Cell viability was assessed after 5 days incubation. (A) Survival is expressed as absorbance in the MTT assay. Effect of paclitaxel can be seen as the change in survival at 0 Gy. (B) Survival curves are presented with survival expressed relative to the unirradiated control treated with the same paclitaxel concentration. Points, mean of triplicate determinations; bars, standard deviation.

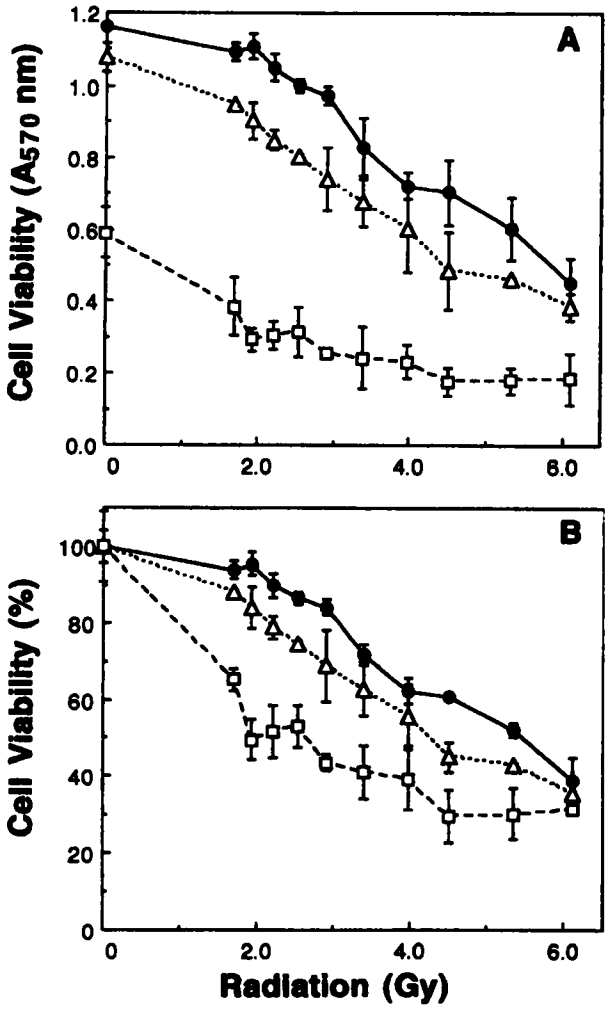

Figure 3. Radiation survival curves of H/E8 subline pretreated with paclitaxel. H/E8 cells were treated for 1 h with 600 nM (△) or 3 μM (□) paclitaxel, and irradiated as described in Figure 2.

μM) also resulted in significant sensitization to 2 Gy radiation (2.3-fold; $p < 0.001$). However, the sensitization to 2 Gy radiation was evident well below the IC_{50} dose of paclitaxel (3085 ± 250 nM) and at 600 nM, which alone had no effect on cell viability (Figure 3a; zero radiation), sensitization was 1.3-fold ($p < 0.05$; Figure 3). Figure 4 demonstrates that in the H/E8 subline, paclitaxel treatment sensitized the cells at or above 600 nM. Thus the drug resistant subline was significantly sensitized to radiation after treatment with paclitaxel for 1 h with drug doses that had minimal cytotoxic effect on the cells. However, if cells were radiated within 2 h of the exposure to paclitaxel rather than 24 h after exposure, then no sensitization resulted (data not shown).

Since the H/E8 subline expresses P-glycoprotein and paclitaxel is effluxed by P-glycoprotein, the effect of verapamil, an inhibitor of P-glycoprotein mediated drug efflux, was examined. Figure 5 shows that the H/H8 subline was sensitized to radiation (up to 1.6-fold) by treatment with paclitaxel doses as low as 30 nM in the presence of 10 μM verapamil ($p < 0.01$ at 2 Gy radiation). Verapamil alone had no effect on the radiation sensitivity of the H/E8 subline (Figure 5) nor did verapamil have any effect on the HL60 cells (results not shown).

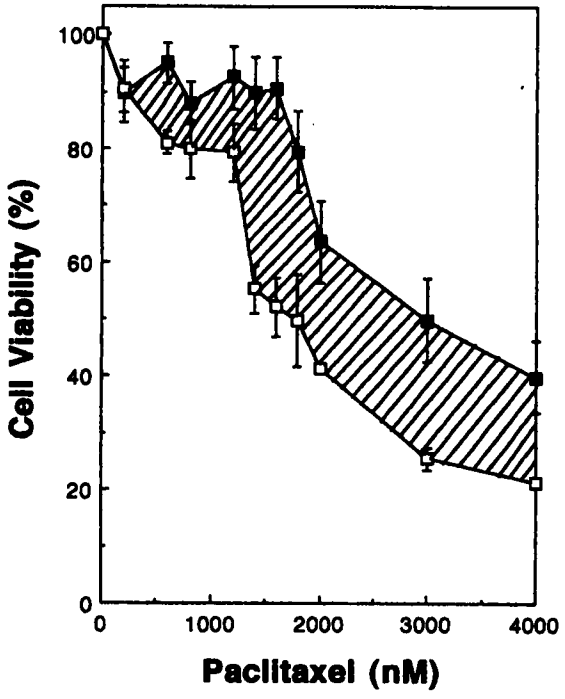

Figure 4. Effect of pretreatment with paclitaxel on cell viability after 2 Gy radiation. Cells were treated for 1 h with a range of paclitaxel concentrations. After 24 h incubation, cells were exposed to 2 Gy radiation and cell viability assessed after 5 days incubation (open symbols). The effect of paclitaxel alone is shown for comparison (closed symbols). Points, mean of triplicate determinations; bars, standard deviation.

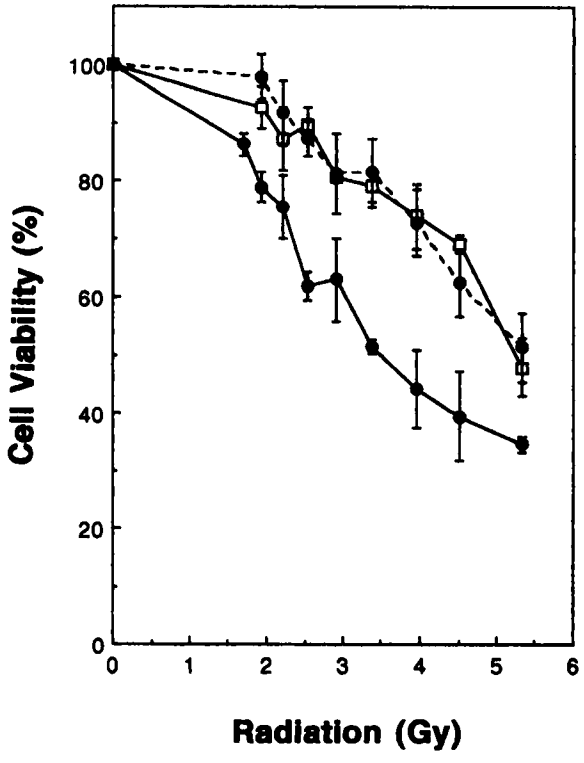

Figure 5. Effect of verapamil on cell survival following combined paclitaxel and radiation treatment. H/E8 were treated with 30 nM paclitaxel (solid symbols) for 1 h, alone (broken line) or in the presence of 10 μM verapamil (solid line). Cell viability was determined after exposure to radiation. The effect of radiation and 10 μM verapamil without paclitaxel pretreatment is shown (open symbols).

Paclitaxel is thought to exert its effect on radiation by blocking cells in the G_2/M phase of the cell cycle, which is known to be the most radiation sensitive phase of cell growth. Examination of the effect of paclitaxel on both the HL60 cells and the H/E8 subline shows that cells accumulated in the G_2/M phase of the cell cycle at a paclitaxel concentration near or above the IC_{50} (Figure 6). At doses below the IC_{50} there was little effect on the cell cycle distribution.

Discussion

The potential of paclitaxel in the clinic has been recognized for some time. The responses observed on treatment with paclitaxel in drug refractory ovarian and breast cancer have caused much optimism for its future use in the treatment of refractory cancer.[5] The unique action of paclitaxel with its ability to block cells in the G_2/M phase of the cell cycle has aroused interest in its use as a radiosensitizing agent, especially for the treatment of refractory disease. Reports on the ability of paclitaxel to sensitize cells to radiation are variable and even the most recent literature shows that while some cell types may be sensitized to radiation treatment by paclitaxel,[21] other cell types show no increased response.[22] The HL60 cell line and the extended multidrug resistant H/E8 subline offer a unique opportunity to examine the effects of radiation and paclitaxel on a cell which displays the type of drug and radiation resistance that is encountered in the treatment of refractory cancer. In the parental HL60 cells, the results here confirm those of Choy *et al.*,[9] who also demonstrated a low, but significant sensitization to 2 Gy radiation (1.4-fold) by paclitaxel treatment for 1 h, 24 h before radiation (Figure 2). While the H/E8 subline expresses P-glycoprotein[17] and is therefore resistant to natural product drugs including paclitaxel, it is also resistant to non-MDR drugs and radiation (Figure 1). However, pretreatment with paclitaxel clearly sensitized the cells to radiation at doses of paclitaxel which alone had little effect on cell viability (Figure 3) suggesting

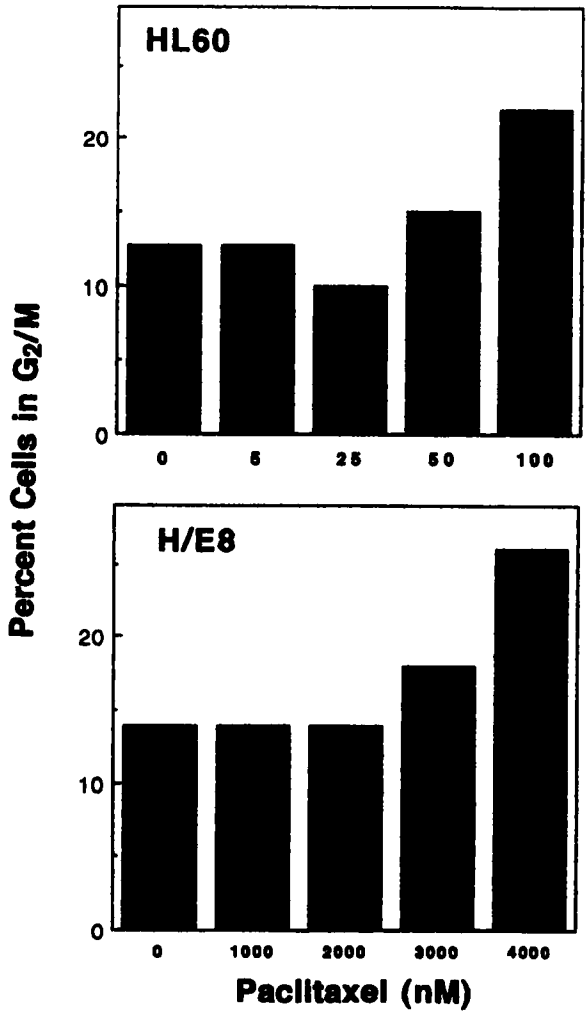

Figure 6. Effect of paclitaxel treatment of cell cycle. HL60 and H/E8 cells were treated with paclitaxel for 1 h and DNA content analyzed by flow cytometry at 24 h after the addition of paclitaxel. Results are the mean of duplicate determinations.

that this combination offers a potential therapeutic advantage in the treatment of refractory tumors.

While a range of paclitaxel and radiation doses were examined, most data is presented at 2 Gy radiation as this is the dose normally administered in a single fractionated treatment. In the drug resistant H/E8 subline, sensitization to 2 Gy radiation could be achieved by pretreatment with 600 nM paclitaxel (Figures 3 and 4). In combination with verapamil, the paclitaxel dose required for sensitization was reduced to 30 nM (Figure 5), 100-fold lower than the dose required for a 50% survival of H/E8 subline treated with paclitaxel alone and well below the toxicity limits for this drug.[23] Therefore these drug and radiation resistant cells are able to be sensitized by paclitaxel at doses similar to those which affect the parental cells. Further, these results suggest that it is necessary for the paclitaxel to enter the cells to exert an effect. Since paclitaxel is normally administered in a 50% Cremophor EL mixture,[24] which has been reported to inhibit P-glycoprotein mediated drug efflux,[25] this would have a similar effect to verapamil in sensitizing P-glycoprotein expressing cells to paclitaxel.

The HL60 cells and the drug resistant subline both demonstrate accumulation of cells in the G_2/M phase of the cell cycle only at concentrations near the IC_{50} dose of paclitaxel. Therefore the sensitization to radiation treatment occurs at paclitaxel concentrations well below the dose required to cause accumulation of cells in the G_2/M phase of the cell cycle (Figure 6). This suggests that the mechanism of radiosensitization involves something other than a G_2/M block. Recently, paclitaxel has been shown to exert its effect at other stages of the cell cycle,[16,26] so it is interesting to speculate on the mechanism by which paclitaxel could sensitize cells to radiation. As paclitaxel binds to tubulin,[27] it may exert its effect indirectly on the repair of damaged DNA after radiation by inhibiting some tubulin-dependent transport system associated with DNA repair. Paclitaxel has also been shown to inhibit DNA synthesis, which also could interfere with DNA repair after radiation.[28] The lack of sensitization by radiation immediately after treatment with paclitaxel, however, confirms that it is not simply a direct effect of paclitaxel. In this regard it is also interesting to note that 2 Gy radiation treatment of both the HL60 cell line and the drug resistant H/E8 subline 24 h prior to a paclitaxel cytotoxicity assay had no effect on the cytotoxicity of paclitaxel (unpublished results).

Conclusion

In vitro studies often demonstrate ways of increasing the efficacy of treatment. Unfortunately this increased efficacy is frequently shown later not to be specific for the malignancy and therefore not to offer any increased therapeutic advantage. This *in vitro* study demonstrates that the radiosensitization by paclitaxel preferentially affects cells expressing the extended multidrug resistance phenotype, a phenotype associated with malignancy. While the mechanism of sensitization to radiation by paclitaxel is unknown, the potentiation of lethal radiation damage by paclitaxel in cells expressing an extended multidrug resistance phenotype suggests

pretreatment with low, tolerable doses of paclitaxel may offer a significant therapeutic advantage in the radiation treatment of refractory tumors.

References

1. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Ann Rev Biochem* 1993; **62**: 385–427.
2. Benchimol S, Ling V. P-glycoprotein and tumor progression. *J Natl Cancer Inst* 1994; **86**: 814–5.
3. Cole SP, Bhardwaj G, Gerlach JH, *et al.*. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992; **258**: 1650–4.
4. Guchelaar H-J, Ten Napel CHH, de Vries EGE, Mulder NH. Clinical, toxicological and pharmaceutical aspects of the antineoplastic drug taxol: a review. *Clin Oncol* 1994; **6**: 40–8.
5. O'Shaughnessy JA, Cowan KH. Current status of paclitaxel in the treatment of breast cancer. *Breast Cancer Res Treat* 1994; **33**: 27–37.
6. Rowinsky EK. Update on the antitumor activity of paclitaxel in clinical trials. *Ann Pharmacother* 1994; **28**: S18–S22.
7. Rowinsky EK, Cazenave LA, Donehower RC. Taxol: a novel investigational antimicrotubule agent. *J Natl Cancer Inst* 1990; **82**: 1247–59.
8. Choy H, Akerley W, Safran H, *et al.* Phase I trial of outpatient weekly paclitaxel and concurrent radiation therapy for advanced non-small-cell lung cancer. *J Clin Oncol* 1994; **12**: 2682–6.
9. Choy H, Rodriguez FF, Koester S, Hilsenbeck S, Von Hoff DD. Investigation of taxol as a potential radiation sensitizer. *Cancer* 1993; **71**: 3774–8.
10. Geard CR, Jones JM, Schiff PB. Taxol and radiation. *Monogr Natl Cancer Inst* 1993; **15**: 89–94.
11. Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB. Changes in radiation survival curve parameters in human tumor and rodent cells exposed to paclitaxel (taxol). *Int J Radiat Oncol Biol Phys* 1994; **29**: 559–64.
12. Steren A, Sevin B-U, Perras J, *et al.* Taxol sensitizes human ovarian cancer cells to radiation. *Gynecol Oncol* 1993; **48**: 252–8.
13. Steren A, Sevin B-U, Perras J, *et al.* Taxol as a radiation sensitizer: a flow cytometric study. *Gynecol Oncol* 1993; **50**: 89–93.
14. Tishler RB, Geard CR, Hall EJ, Schiff PB. Taxol sensitizes human astrocytoma cells to radiation. *Cancer Res* 1992; **52**: 3495–7.
15. Tishler RB, Schiff PB, Geard CR, Hall EJ. Taxol: a novel radiation sensitizer. *Int J Radiat Oncol Biol Phys* 1992; **22**: 613–7.
16. Minarik L, Hall EJ. Taxol in combination with acute and low dose rate irradiation. *Radiother Oncol* 1994; **32**: 124–8.
17. Su GMI, Davey MW, Davey RA, Kidman AD. Development of extended multidrug resistance in HL60 promyelocytic leukemia cells. *Br J Haematol* 1994; **88**: 566–74.
18. Gallagher R, Collins S, Trujillo J, *et al.* Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with promyelocytic leukemia. *Blood* 1979; **54**: 713–33.
19. Marks DC, Belov L, Davey MW, Davey RA, Kidman AD. The MTT cell viability assay for cytotoxicity testing in multidrug resistant human leukemic cells. *Leukemia Res* 1992; **16**: 1165-73.
20. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mithcell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensivity. *Cancer Res* 1987; **47**: 943–6.
21. Rodriguez M, Sevin BU, Perras J, *et al.* Paclitaxel—a radiation sensitizer of human cervical cancer cells. *Gynecol Oncol* 1995; **57**: 165–9.
22. Stromberg JS, Lee YJ, Armour EP, Martinez AA, Corry PM. Lack of radiosensitization after paclitaxel treatment of three human carcinoma cell lines. *Cancer* 1995; **75**: 2262–8.
23. Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E. Phase 1 clinical and pharmacokinetic study of taxol. *Cancer Res* 1987; **47**: 2486–93.
24. Webster L, Linsenmeyer M, Millward M, Morton C, Bishop J, Woodcock D. Measurement of cremophor EL following taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug resistant phenotype. *J Natl Cancer Inst* 1993; **85**: 1685–90.
25. Woodcock DM, Jefferson S, Linsenmeyer ME, *et al.* Reversal of multidrug resistance phenotype with cremophor EL, a common vehicle for water-insoluble vitamins and drugs. *Cancer Res* 1990; **50**: 4199–203.
26. Long BH, Fairchild CR. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. *Cancer Res* 1994; **54**: 4355–61.
27. Roberts JR, Rowinsky EK, Donehower RC, Robertson J, Allison DC. Demonstration of the cell cycle positions of taxol induced 'asters' and 'bundles' by sequential measurements of tubulin immunofluorescence, DNA content, and autoradiagraphic labelling of taxol-sensitive and resistant cells. *J Histochem Cytochem* 1989; **37**: 1659–65.
28. Ball RL, Carney DH, Albrecht T. Taxol inhibits stimulation of cell DNA synthesis by human cytomegalovirus. *Exp Cell Res* 1990; **191**: 37–44.

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