Modulation of drug and radiation resistance in small cell lung cancer cells by paclitaxel

Vicki L. Locke[a], Ross A. Davey[b] and Mary W. Davey[a]

Small cell lung cancer (SCLC) responds to treatment with cisplatin and etoposide, but relapse is rapid and survival rates are low. Our aims were to determine the mechanisms of resistance and the potential for paclitaxel (Taxol) to overcome any drug or radiation resistance. To mimic clinical treatment, H69 SCLC cells, representative of the classic form of the disease, and H82 cells, with the phenotype of the more resistant variant disease, were treated intermittently with 100 ng/ml cisplatin or 500 ng/ml etoposide (approximate IC_{50} drug doses) to produce stable sublines. Drug and radiation resistance were determined using the MTT assay. Protein expression was determined by Western blot. The effect of paclitaxel on drug resistance was determined by cytotoxicity assays. Intermittent 4-day treatment with 100 ng/ml cisplatin caused 2- to 3-fold resistance to cisplatin ($n=5$; $p<0.05$), and 2- to 5-fold cross resistance to etoposide, alkylating drugs, the Vinca drugs and radiation. Resistance was mediated primarily by changes in glutathione metabolism and was not associated with changes in MRP2 transport protein. Treatment with etoposide (500 ng/ml) produced cells with 2-fold resistance to etoposide ($n=5$; $p<0.05$). Cross-resistance was limited and mediated by decreased topoisomerase IIα. Treatment of both drug-resistant sublines with a maximal non-cytotoxic dose of paclitaxel sensitized them to other drugs and to radiation, although this treatment had no effect on the parental H69 or H82 cells. We conclude that paclitaxel may play an important role in the treatment of refractory SCLC. *Anti-Cancer Drugs* 14:523–531 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:523–531

Keywords: cisplatin, drug resistance, etoposide, glutathione, paclitaxel (Taxol) sensitization, radiation resistance

[a]Department of Cell and Molecular Biology, University of Technology, Sydney, Australia and [b]Bill Walsh Cancer Research Laboratories, Royal North Shore Hospital, St Leonards NSW, Australia.

Correspondence to M. Davey, Department of Cell and Molecular Biology, University of Technology, Sydney, Westbourne Street, Gore Hill, NSW 2065 Australia.
Tel: +61 2 9514 4065; fax: +61 2 9514 4026;
e-mail: Mary.Davey@uts.edu.au

Received 3 March 2003 **Revised form accepted** 21 May 2003

Introduction

Lung cancer is a leading cause of cancer-related deaths and remains a world-wide public health problem. Small cell lung cancer (SCLC) accounts for 20–25% of lung cancer and is one of the most aggressive forms of the disease. The principal treatment for all stages of SCLC is combination chemotherapy with high response rates and improved survival, although patients with the classic form of SCLC respond to treatment better than those with the variant form of the disease [1]. However, relapse and disease progression occurs in the majority of patients. With the introduction of radiotherapy and new drug combinations, the 5-year survival for limited-stage disease has improved in recent years to 24% [2]. However, the 5-year survival for all patients is still only about 5% with little improvement since the introduction of combination chemotherapy in the early 1970s [3,4]. The most significant reason for the failure of drug therapy is the development of drug resistance. *In vitro* studies have identified several resistance mechanisms involved in multidrug resistance (MDR), the most commonly described mechanisms being the expression of drug resistance proteins, P-glycoprotein, multidrug resistance-associated protein (MRP1) and breast cancer resistance protein (BCRP). These proteins are members of the ATP-binding cassette superfamily of transport proteins which confer resistance to a wide range of natural product drugs including anthracyclines and etoposide, commonly used in the treatment of SCLC [5].

The failure of chemotherapy in SCLC has led to the introduction of new drugs in combination with the standard treatment with anthracyclines, etoposide and/or cisplatin, in an attempt to improve treatment outcomes [3]. Paclitaxel, an antimitotic drug, has been successful in the treatment of many drug-resistant cancers [6] and shows improved response in combination with cisplatin/etoposide treatment [7]. While the cytotoxic activity of paclitaxel is associated with enhanced assembly and stability of microtubules, paclitaxel has many effects on cellular metabolism [8–10]. To exploit the success of paclitaxel and improve the survival rate of lung cancer patients requires an understanding of the action of these drugs so that treatment protocols may be optimized for drug dose and timing.

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000086841.52271.75

Pre-clinical testing of drugs in cultured cells provides a simple method to determine potential drug interactions or synergies as these *in vitro* models retain many properties of the tumor from which they were derived [11]. However, many studies on cellular resistance use drug doses far above those able to be achieved clinically and their relevance must be questioned. In contrast, treatment of SCLC cells with low doses of an anthracycline showed drug resistance developed to a wide range of drugs, not simply to the MDR, natural product drugs [12,13]. Although the drug-resistant SCLC cells were 2- to 10-fold resistant to most drugs, treatment with paclitaxel reversed the resistance and sensitized the cells to many drugs including cisplatin and etoposide [12,13]. As cisplatin and etoposide are the most commonly used drugs to treat SCLC [4], we have extended our studies to examine the development of drug resistance to cisplatin and etoposide in SCLC cells, and the ability of paclitaxel to modulate this resistance.

Materials and methods

Cell cultures

The H69 and H82 human SCLC cells were from ATCC (Rockville, MD). Cells and sublines were grown in RPMI1640 (Trace Biosciences, Sydney, Australia) containing 10% fetal bovine serum (Trace Biosciences), 20 mM HEPES and 10 mM $NaHCO_3$ in a 5% CO_2 atmosphere at 37°C. All cells and sublines were free of *Mycoplasma* and exponentially growing cells were used for all experiments.

To mimic clinical treatment, the H69-CP and H82-CP sublines were developed by treating either the H69 or H82 cells with 100 ng/ml of cisplatin for 4 days with a recovery period of 2 weeks, for a total of six treatments. The H69-VP and H82-VP sublines were similarly developed using 500 ng/ml of etoposide. The etoposide-resistant cells were then exposed to 1 μg/ml for further six treatments to develop the H69-VP1 and H82-VP1. The resistance of these sublines was constant for over 3 months in the absence of drug treatment. However, to ensure the stability of the sublines, cells were treated with the appropriate drug dose every 8–10 weeks.

Cytotoxicity assays

Etoposide, genistein, chlorambucil and paclitaxel (Taxol) were purchased from Sigma (St Louis, MO). Epirubicin and daunorubicin were from Pharmacia (Sydney, Australia), vinblastine, navelbine and cisplatin were from David Bull Laboratories (Sydney, Australia), and amsacrine was kindly provided by Professor Bruce Baguley (University of Auckland Medical School, New Zealand).

Sensitivity to drugs was determined using the MTT (Sigma) cell viability assay. Cells (6×10^4/well for H69 cells and sublines and 5×10^4/well for H82 cells and sublines) were incubated in triplicate in 96-well plates with 2-fold serial drug dilutions for 5 days [14]. The 50% inhibitory concentration (IC_{50}) was determined as the drug concentration resulting in a 50% reduction in cell viability. Relative resistance was calculated by dividing the IC_{50} obtained for the resistant subline by the IC_{50} obtained for the parental cell line. For modulation of resistance by paclitaxel, cells (5×10^5/ml) were exposed to the drug (10 ng/ml) for 1 h. The cells were resuspended in fresh media and incubated for 24 h before drug resistance was tested using the MTT assay.

Radiation sensitivity was determined by the MTT assay, similar to the drug sensitivity. Cells were exposed to a linear gradient of X-radiation using a 6-MV X-ray linear accelerator treatment machine fitted with a linear field lead wedge. Control wells were not irradiated. Plates were incubated for 5 days and cell viability determined as above.

Western blot analysis

Cells (5×10^6) were washed in phosphate buffered saline (pH 7.2), and resuspended in 100 μl lysis buffer (50 mM Tris, 1 mM PMSF), sonicated and 20 μg total protein was subjected to electrophoresis on Tris–glycine 4–20% gradient gel (Gradipore, Sydney, Australia). Blots were developed using antibodies to topoisomerase (Topo) IIα, Topo 1 (TopoGEN, Columbus, OH), glutathione-S-transferase π (GSTπ; Sigma), at a 1:500 dilution. Membranes were re-developed using anti-β-actin (Sigma), and protein was quantitated using the Microtek ScanMaker III and the Molecular Analyst program (Bio-Rad, Hercules, CA). For MRP1 and P-glycoprotein expression, membrane fractions were prepared [13] and subjected to electrophoresis as above. Blots were developed for P-glycoprotein, using C219 antibody (Centacore, Malvern, PA) at a dilution of 1:500 and MRP1, using B5 antibody (kindly provided by Dr D Keppler, Heidelberg, Germany) at a 1:1000 dilution.

Topo II activity assay

The etoposide-dependent formation of cleavable complexes was determined as the amount of radioactivity co-precipitated by K^+/SDS as previously described [15].

Cellular glutathione

Total intracellular glutathione was determined by the colorimetric method using glutathione reductase [16].

Statistical analysis

All experiments were repeated at least twice and significance determined on at least two independent experiments using the Student's *t*-test. Most analysis used three to five replicates and numbers are indicated in the results.

Results

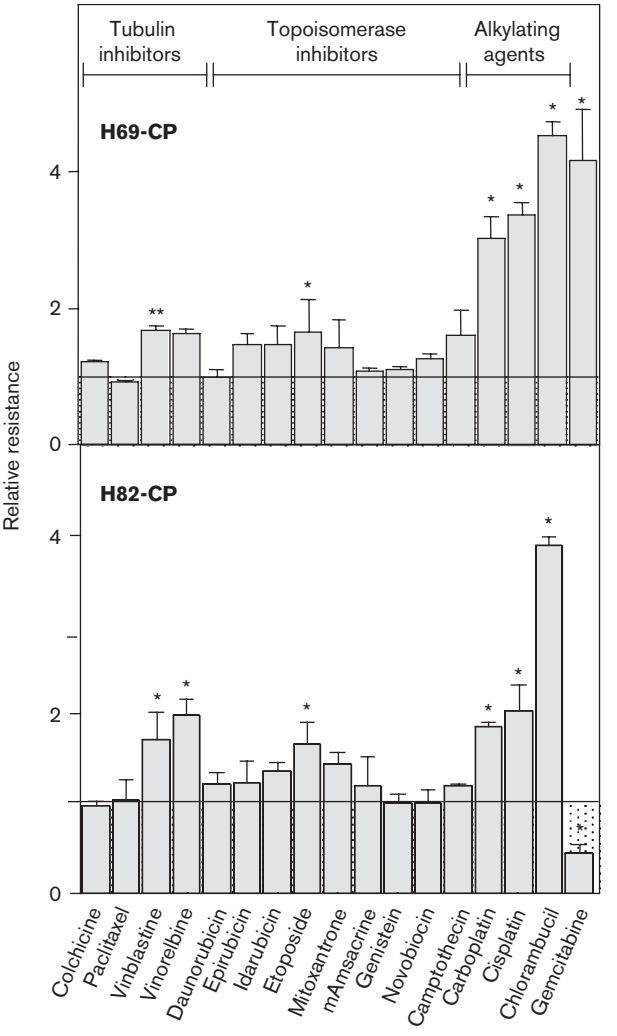
Development of drug resistance

To examine cisplatin resistance, H69 cells, representative of the 'classic' form of SCLC, and H82 cells, representative of the more drug-resistant 'variant' form of SCLC, were treated for 4 days with 100 ng/ml cisplatin. This dose was chosen since the IC_{50} for cisplatin in a 4-day cytotoxicity assay is 140 ± 1 ng/ml ($n = 3$) for both the H69 and H82 cells. After six treatments with a 2-week recovery between treatments, the growth kinetics of the cells were unaffected by drug exposure. Treatment produced the H69-CP with 3-fold resistance to cisplatin ($n = 5$; $p < 0.05$) and the H82-CP with 2-fold resistance to cisplatin ($n = 5$; $p < 0.05$). Both sublines showed a similar cross-resistance profile (Fig. 1) with highest resistance to the alkylating agents (2- to 5-fold) and low, but significant resistance (2-fold; $p < 0.05$) to the tubulin inhibitors vinblastine and vinorelbine, and to the Topo inhibitor etoposide. However, while the H69-CP cells were 3.8-fold resistant to the antimetabolite gemcitabine ($p < 0.05$), the H82-CP were 2-fold sensitized to gemcitabine ($p < 0.05$). The sublines retained this resistance for more than 3 months (longer times were not tested).

Figure 2 shows that treatment with etoposide (500 ng/ml) resulted in the H69-VP with 2-fold resistance to the selecting drug ($n = 5$; $p < 0.05$) and the H82-VP which were 3-fold resistant to etoposide ($n = 5$; $p < 0.05$). Both sublines were resistant to mitoxantrone (2- to 3-fold). In addition, the H69-VP cells were resistant to the Topo II activity inhibitor novobiocin (1.5-fold; $p < 0.05$), but were significantly sensitized to the tubulin inhibitors vinblastine (2.6-fold; $p < 0.05$) and vinorelbine (2-fold; $p < 0.05$). In contrast, the H82-VP also showed significant resistance to the anthracyclines, epirubicin and idarubicin, and the other Topo inhibitors amsacrine and genistein (Fig. 2). As the resistance in these sublines was low, they were subsequently treated with 1 µg/ml etoposide as described in the methods, to produce the H69-VP1 and the H82-VP1 sublines. This treatment had little effect on cell growth, but the H69-VP1 subline showed increased resistance to etoposide (10-fold; $p < 0.05$), epirubicin (3.5-fold; $p < 0.05$) and cisplatin (5-fold; $p < 0.05$). However, cells were no longer sensitive to the Vinca drugs. There was little increase in resistance for the H82-VP1 subline.

As current treatment protocols for SCLC usually include radiation, the response to radiation was determined in the drug-resistant sublines. The H69-CP showed increased resistance to radiation (Fig. 3), while no resistance to radiation developed in the H69-VP cells. The radiation resistance of the H82 cells was significantly higher than the H69 cells and there was no significant change in the drug-resistant sublines (not shown).

Fig. 1

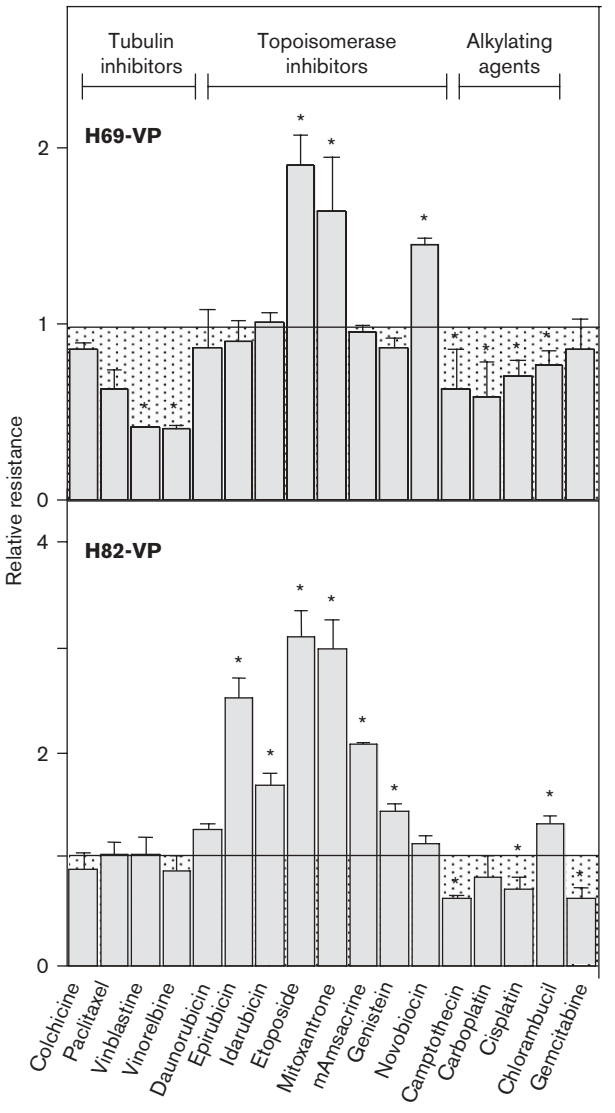


Drug resistance profile of the H69-CP and H82-CP sublines. Drug resistance was determined by exposing cells to drug for 5 days after which the cell viability was determined by the MTT assay and fold resistance was calculated. The stippled area (bordered by a line) indicates the relative resistance of 1 for the parental cells. Error bars represent SDs of the IC_{50} determined in three or more independent experiments and an asterisk indicates a significant change ($p \leq 0.05$) compared to the parental cells using Student's *t*-test.

Resistance mechanisms

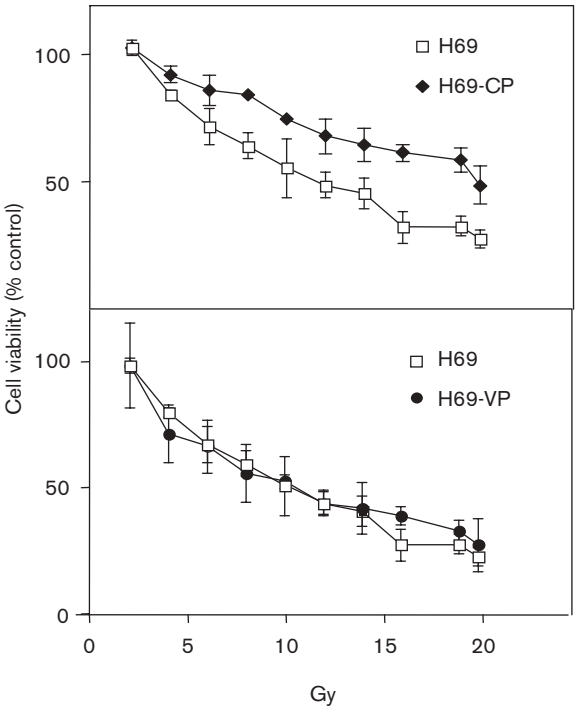
Since all the drug-resistant sublines were resistant to etoposide, and Topo II is the target for this drug, Topo II expression was determined. Figure 4(A) shows that while there was no change in Topo IIα expression in the cisplatin-resistant sublines, expression was significantly reduced in the etoposide-treated sublines. This correlated with a decrease in etoposide-dependent cleavable complex formation (Fig. 4B), demonstrating that etoposide resistance in the H69-VP and H82-VP sublines was mediated through changes in Topo IIα. As has been

Fig. 2



Drug resistance profile of the H69-VP and H82-VP sublines. Drug resistance was determined as in Fig. 1. The stippled area (bordered by a line) indicates the relative resistance of 1 for the parental cells. Error bars represent SDs of the IC_{50} determined in three or more independent experiments and an asterisk indicates a significant change ($p \leq 0.05$) compared to parental cells using Student's *t*-test.

Fig. 3



Sensitivity to radiation in the H69 cells and drug-resistant cell lines. Sensitivity of the H69 (□), H69-CP (♦) and H69-VP (●) cells was determined by exposure to a range of radiation doses. Cell viability was determined by the MTT assay after 5 days incubation. Points are means of triplicate wells; error bars are the SD of the triplicate wells. Representative of three independent assays.

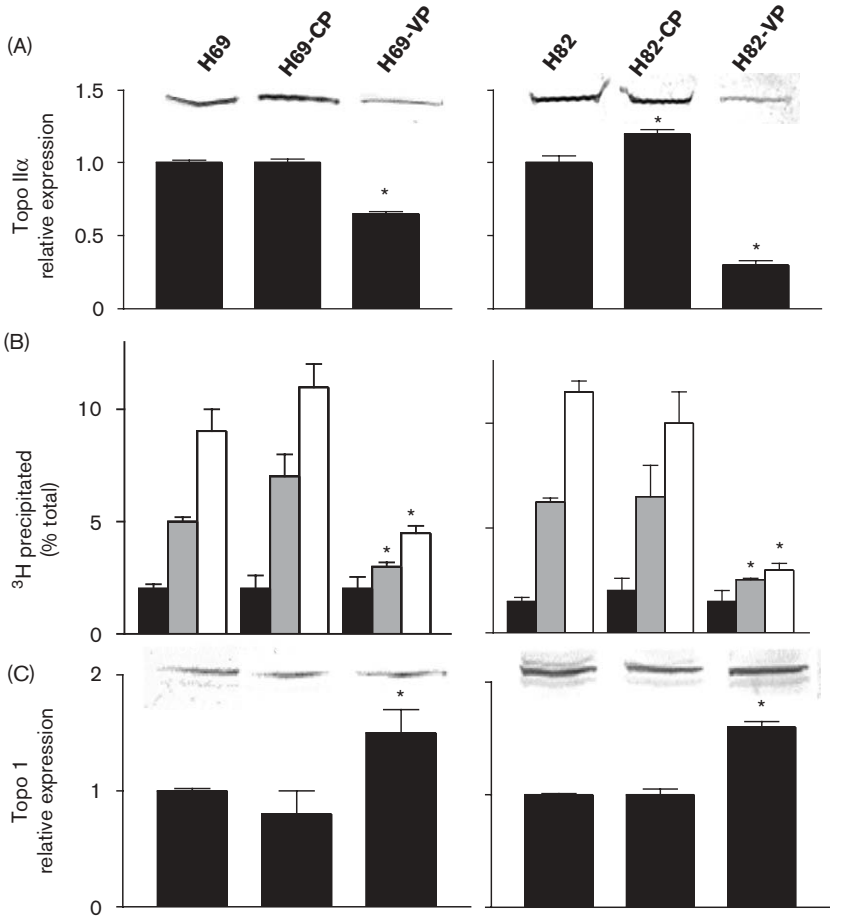
observed for other drug-resistant cells, the decrease in Topo IIα expression correlated with increases in Topo I expression in the H69-VP (1.7-fold; $p < 0.01$) and H82-VP sublines (1.6-fold; $p < 0.01$; Fig. 4C). This increase could account for the increased sensitivity of these sublines to the Topo I drug, camptothecin (Fig. 2; 2-fold sensitized; $p < 0.05$).

Although changes in Topo IIα would explain resistance to Topo II inhibitors, this could not explain the cross-resistance to other drugs, nor does it explain the resistance to etoposide in the cisplatin-treated cells. While the multidrug-transport proteins P-glycoprotein and MRP1 confer resistance to etoposide by decreasing the intracellular drug accumulation [16], there was no change in expression of these proteins in any of the sublines (not shown). Nor were there any changes in MRP2, which confers resistance to cisplatin (not shown). However, GSTπ was significantly increased in the H69-CP (1.8-fold; $p < 0.05$) and H82-CP (1.3-fold; $p < 0.05$) sublines, potentially accounting for their resistance to the alkylating drugs (Fig. 5A). Correlating with the increased GSTπ was a significant increase in cellular glutathione (Fig. 5B). Interestingly the H82-VP subline had a low, but significant increase in cellular glutathione (120%; $n = 3$; $p < 0.05$), which could account for the increased cross-resistance in these cells compared to the H69-VP subline which showed no changes in glutathione.

Effect of paclitaxel treatment on drug-resistant cells

Previously we have found that treatment of epirubicin-resistant cells (H69-EPR; H82/E8) with a non-cytotoxic dose of paclitaxel for 1 h (10 ng/ml) was able to sensitize

Fig. 4



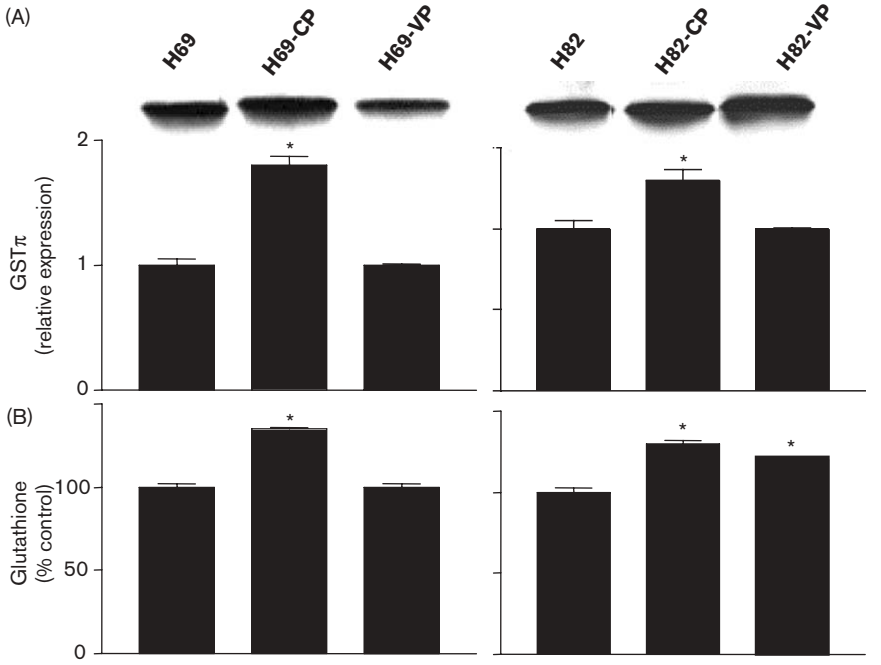
Topoisomerase expression. (A and C) Total cell extracts were prepared and 40 μg protein was electrophoresed on 4–20% SDS polyacrylamide gels, electrotransferred and membranes probed with anti-Topo IIα antibody (A) or anti-Topo I antibody (C). Membranes were then probed with anti-β-actin antibody. Results are presented as expression relative to β-actin, determined using Molecular Analyst software, from three independent experiments. An asterisk indicates significantly changed expression compared to the parental cells ($p < 0.01$). (B) Cleavable complex formation was quantified by the K^+/SDS precipitation assay after 1 h incubation without etoposide (solid bars) or with 7.5 ng/ml etoposide (hatched bars) or 15 ng/ml etoposide (open bars) as described in Materials and methods. The amount of co-precipitated [^{3}H]DNA is expressed as percent of the total radioactivity in the lysate. Results are the mean of three independent experiments, performed in duplicate, and error bars represent SD. An asterisk indicates significantly reduced etoposide-induced cleavable complexes compared to the parental cells at the same drug concentration ($p < 0.05$, Student's *t*-test).

the cells to other drugs [12,13]. This treatment with paclitaxel has no effect on viability of the parental or the drug-resistant cells nor does it affect the distribution of cells in the cell cycle [12]. Paclitaxel treatment of the H69-CP and H69-VP cells (Fig. 6) was able to sensitize both drug-resistant sublines to cisplatin or etoposide, respectively, while this treatment had no effect on the parental H69 cells. The H69-CP cells were also sensitized to chlorambucil (2.4-fold; $p < 0.05$) and etoposide (1.6-fold; $p < 0.05$). Importantly, paclitaxel treatment was also able to sensitize the H69-CP cells to radiation (Fig. 6). Paclitaxel treatment similarly enhanced the sensitivity of the H82-CP (1.9-fold; $p < 0.05$) and H82-VP (1.7-fold; $p < 0.05$) to cisplatin and etoposide, respectively. The H82-CP cells were also sensitized to chlorambucil (2.9-fold; $p < 0.05$) and etoposide (1.8-fold; $p < 0.05$), while the H82-VP subline was sensitized to epirubicin (1.7-fold; $p < 0.05$).

Discussion

Treatment of lung cancer presents a significant clinical problem since the initial response to treatment is rapidly followed by relapse and resistance to further treatment. The results here demonstrate that drug resistance develops readily in response to treatment with very low doses of drugs used in the treatment of SCLC. In contrast to the broad drug resistance, or an extended MDR phenotype, induced in the H69 and H82 cells by

Fig. 5



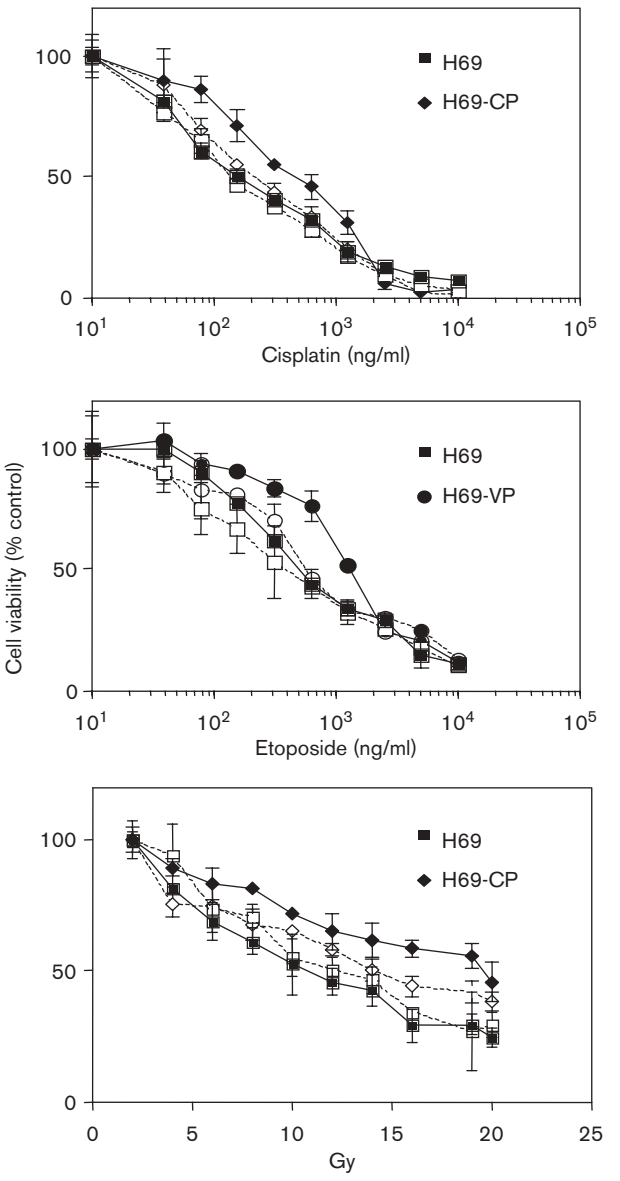
Expression of GSTπ and total intracellular glutathione. (A) Total cell extracts were prepared and electrophoresed on 4–20% SDS polyacrylamide gels, electrotransferred and membranes probed with anti-GSTπ. The expression of GSTπ relative to β-actin was determined using Molecular Analyst software and results are the mean of two independent experiments. An asterisk indicates significantly increased ($p<0.05$) expression compared to the parental cells. (B) Exponentially growing cells were assayed for glutathione using a kinetic assay. Results are the mean of two independent experiments in duplicate and are calculated as percent control; error bars are the SD. An asterisk indicates significantly increased cellular glutathione levels compared to parental cells ($p<0.05$) using Student's *t*-test.

treatment with epirubicin [12,13], resistance induced by cisplatin was mediated by changes in glutathione, resulting in resistance to alkylating agents and Vinca alkaloids. This resistance was reversed by treatment of cells with buthionine sulfoximine, a specific inhibitor of glutathione synthesis (data not shown), confirming the requirement for glutathione synthesis for drug resistance [16]. The development of resistance following treatment with etoposide correlated with decreased Topo IIα expression. Cross-resistance was significantly more limited than that resulting from treatment with cisplatin or epirubicin, and this may account for the initial success of etoposide in the treatment of SCLC [3]. However, treatment with the higher dose of etoposide (1 μM) significantly increased the drug resistance of the more sensitive H69 cells, which could account for the resistance of SCLC at relapse.

Although the H82 cells have different molecular and phenotypic characteristics compared to the classic H69 cells [17], the pattern of resistance induced by drug treatment was similar in both cell types. Cisplatin treatment produced sublines with resistance mediated by increased glutathione and increased GSTπ. Cellular glutathione appears to be the rate-limiting step in detoxification of cisplatin [18], although other resistance mechanisms such as decreased drug accumulation resulting from decreased uptake or increased efflux or changes in apoptotic response, may also be clinically important [19]. Cisplatin resistance has also been associated with increased MRP2 expression [20,21]. However, the cisplatin-resistant lung cells did not have increased MRP2, consistent with reports that in lung tissue from patients treated with cisplatin, γ-glutamylcysteine synthase and GSTπ were increased, but MRP2 was unchanged [22,23]. The increased radiation resistance of the H69-CP is also consistent with increased expression of GSTπ, since transfection studies have shown that increased GSTπ confers radiation resistance [24].

The H82-CP also showed an increase in Topo IIα, although this did not correlate with a significant increase in the formation of etoposide-dependent cleavable complexes or increased sensitivity to etoposide compared to the H82 cells. Increased Topo IIα has been previously demonstrated after cisplatin treatment [25,26] and this may be a consequence of its role in increasing DNA repair [27]. As no increase in Topo IIα occurred in the H69-CP

Fig. 6



Effect of paclitaxel on resistant cells. H69 and H69 drug-resistant cells were exposed to 10 ng/ml paclitaxel for 1 h, then incubated overnight in drug-free media. The cytotoxicity of cisplatin or etoposide was then assessed by exposure of cells to drug for 5 days (paclitaxel treated, open symbols; control, closed symbols). Sensitivity to radiation was determined after exposure to a linear radiation dose, followed by a 5-day incubation. The cell viability was determined by the MTT assay. Points are means of triplicate wells; error bars indicate the SD. Representative of three independent assays.

cells, increased Topo IIα is not a consistent outcome of cisplatin treatment.

In contrast to the H69-CP, the H69-VP showed decreased Topo IIα expression and decreased ability to form etoposide-dependent cleavable complexes (Fig. 4), and this was associated with limited low-level resistance only to etoposide, mitoxantrone and novobiocin (Fig. 2). The Topo IIα expression was further reduced in the H69-VP1 cells (not presented) correlating with increased resistance to Topo IIα-targeting drugs. Changes in etoposide accumulation have been associated with etoposide resistance and Brock *et al.* [28] reported resistance in etoposide-treated H69 cells was due to increased MRP1 expression. However, this was only detected after treatment with 1.5 μM etoposide, a higher dose than used here. The development of atypical Topo II-associated MDR appears to be an early event in response to low levels of drug, since similar changes have been described in several low-level drug-resistant cell lines [29–32].

Increased sensitivity to the tubulin-inhibiting drugs, as in the H69-VP subline (Fig. 2), is frequently associated with resistance mediated by changes in Topo IIα, although no specific mechanisms for this collateral sensitivity was identified [33]. In contrast to the H69-VP cells, the H69-VP1 cells (not shown) and H82-VP cells (Fig. 2) did not show sensitivity to the tubulin-targeting drugs. Collateral sensitivity to gemcitabine (2-fold) was evident in both etoposide-treated sublines and this is frequently associated with the development of an atypical MDR phenotype, although the underlying mechanism for this association is not known [34,35].

Although different drug resistance proteins were induced in response to cisplatin or etoposide treatment, paclitaxel was able to sensitize the drug-resistant cell lines to the selecting drug and also to radiation (Fig. 6). Similar to its effect on epirubicin-resistant cells [12,13], paclitaxel treatment reversed resistance to cisplatin, chlorambucil, epirubicin and etoposide. It is not surprising when reversal of resistance is achieved by specifically targeting a resistance mechanism, such as glutathione depletion or blocking drug transport pumps. However, as multiple cellular changes have contributed to resistance in these sublines, that paclitaxel was able to reverse this wide range of resistance mechanisms holds great promise for its clinical use. Further, the effect of paclitaxel on drug and radiation sensitivity was specific for the drug-resistant sublines, as paclitaxel had no effect on the sensitivity of the parental H69 or H82 cells. This was also true for the H69-EPR and H82/E8 cells. Paclitaxel also sensitized drug- and radiation-resistant HL60 leukemia cells, showing the effect is not cell-type specific [36]. This apparent specificity for drug-resistant cells has important clinical implications for the treatment of resistant disease.

Paclitaxel has multiple concentration-dependent effects on cellular metabolism, and its ability to reverse such a broad spectrum of resistance mechanisms suggests paclitaxel is not directly interfering with each specific

resistance mechanism in the H69 and H82 drug-resistant sublines. Although several studies have shown that microtubule inhibitors can alter the intracellular transport and accumulation of etoposide, anthracyclines and cisplatin [37–39], but this remains controversial [40–43]. As there is no resistance to paclitaxel in the drug-resistant cells (Figs 1 and 2), this effect is not simply due to changes in paclitaxel accumulation. Our current studies aim to identify the protein changes in response to low-dose paclitaxel in drug-resistant cells.

Studies with cell lines serve as an initial screen for agents able to modulate drug resistance, and these studies reveal potential mechanisms of resistance in SCLC and provide models to test drug combinations. The results show that multiple resistance mechanisms are readily induced in SCLC cells in response to treatment with low drug doses. Importantly, paclitaxel pretreatment was able to overcome this induced resistance and sensitize the resistant sublines. Understanding the mechanism involved in paclitaxel sensitization of drug-resistant lung cancer cells may lead to improved clinical treatment of refractory cancer.

References

1 Volm M, Mattern J. Resistance mechanisms and their regulation in lung cancer. *Crit Rev Oncol* 1996; **7**:227–244.
2 Sherman CA, Lima CMR, Turrisi AT. Limited small-cell lung cancer: a potentially curable disease. *Oncology* 2000; **14**:1395–1403.
3 Kelly K. New chemotherapy agents for small cell lung cancer. *Chest* 2000; **117**:156S–162S.
4 Worden FP, Kalemkerian GP. Therapeutic advances in small cell lung cancer. *Exp Opin Invest Drugs* 2000; **9**:565–579.
5 Tan B, Piwnica-Worms D, Ratner L. Multidrug resistance transporters and modulation. *Curr Opin Oncol* 2001; **12**:450–458.
6 Choy H. Taxanes in combined modality therapy for solid tumors. *Crit Rev Oncol Hematol* 2001; **37**:237–247.
7 Bunn PA. Defining the role of paclitaxel in lung cancer—summary of recent studies and implications for future directions. *Semin Oncol* 1997; **24**:153–162.
8 Wang YR, Duncan R, Ann DK, Hamm-Alvarez SF. Identification of a novel taxol-sensitive kinase activity associated with the cytoskeleton. *Biochem Biophys Res Commun* 2000; **277**:525–530.
9 Yeung TK, Germond C, Chen XM, Wang ZX. The mode of action of Taxol: apoptosis at low concentration and necrosis at high concentration. *Biochem Biophys Res Commun* 1999; **263**:398–404.
10 Torres K, Horwitz SB. Mechanisms of taxol-induced cell death are concentration dependent. *Cancer Res* 1998; **58**:3620–3626.
11 Wistuba II, Bryant D, Behrens C, Milchgrub S, Virmani AK, Ashfaq R, *et al.* Comparison of features of human lung cancer cell lines and their corresponding tumors. *Clin Cancer Res* 1999; **5**:991–1000.
12 Locke VL, Davey RA, Davey MW. Paclitaxel sensitization of multidrug-resistant cells to chemotherapy is independent of the cell cycle. *Cytometry* 2001; **43**:170–174.
13 Su GM, Davey MW, Davey RA. Induction of broad drug resistance in small cell lung cancer cells and its reversal by paclitaxel. *Int J Cancer* 1998; **76**:702–708.
14 Marks DC, Davey MW, Davey RA, Kidman AD. The MTT cell viability assay for cytotoxicity testing in multidrug resistant human leukemic cells. *Leukemia Res* 1992; **16**:1165–1173.
15 Locke VL, Davey RA, Davey MW. Altered drug sensitivity in response to idarubicin treatment in K562 human leukaemia cells. *Br J Haematol* 1999; **106**:86–91.
16 Grech KV, Davey RA, Davey MW. The relationship between modulation of MDR and glutathione in MRP-overexpressing human leukaemia cells. *Biochem Pharmacol* 1998; **55**:1283–1289.
17 Junker K, Wiethege T, Muller KM. Pathology of small-cell lung cancer. *J Cancer Res Clin Oncol* 2000; **126**:361–368.
18 Zhang K, Chew M, Yang EB, Wong KP, Mack P. Modulation of cisplatin cytotoxicity and cisplatin-induced DNA cross-links in HepG2 cells by regulation of glutathione-related mechanisms. *Mol Pharmacol* 2001; **59**:837–843.
19 Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. *Mutat Res* 2001; **478**:23–43.
20 Kauffmann HM, Keppler D, Kartenbeck J, Schrenk D. Induction of cMrp/cMoat gene expression by cisplatin, 2-acetylaminofluorene, or cycloheximide in rat hepatocytes. *Hepatology* 1997; **26**:980–985.
21 Minamino T, Tamai M, Itoh Y, Tatsumi Y, Nomura M, Yokogawa K, *et al. In vivo* cisplatin resistance depending upon canalicular multispecific anion transporter (cMOAT). *Jpn J Cancer Res* 1999; **90**:1171–1178.
22 Oguri T, Fujiwara Y, Katoh O, *et al.* Glutathione S-transferase-pi gene expression and platinum drug exposure in human lung cancer. *Cancer Lett* 2000; **156**:93–99.
23 Oguri T, Fujiwara Y, Isobe T, Katoh O, Watanabe H, Yamakido M. Expression of γ-glutamylcysteine synthetase (γ-GCS) and multidrug resistance-associated protein (MRP), but not human canalicular multispecific organic anion transporter (cMOAT), genes correlates with exposure of human lung cancers to platinum drugs. *Br J Cancer* 1998; **77**:1089–1096.
24 Miyara H, Hida T, Nishida K, Takahashi T, Sugiura T, Ariyoshi Y, *et al.* Modification of chemotherapy-radiosensitivity of a human lung cancer cell line by introduction of the glutathione S-transferase π gene. *Jpn J Clin Oncol* 1996; **26**:1–5.
25 Kikuchi Y, Hirata J, Yamamoto K, Ichii K, Kita T, Kudoh K, *et al.* Altered expression of γ-glutamylcysteine synthetase, metallothionein and topoisomerase I or II during acquisition of drug resistance to cisplatin in human ovarian cancer cell. *Jpn J Cancer Res* 1997; **88**:213–217.
26 Jain N, Lam Y-M, Pym J, Campling BG. Mechanisms of resistance of human small cell lung cancer lines selected in VP-16 and cisplatin. *Cancer* 1996; **77**:1797–1808.
27 Gaffney DK, Lundquist M, Warters RL, Rowley R. Effects of modifying topoisomerase II levels on cellular recovery from radiation damage. *Radiat Res* 2000; **154**:461–466.
28 Brock I, Hipfner DR, Nielsen BS, Jensen PB, Deeley RG, Cole SPC, *et al.* Sequential coexpression of the multidrug resistance genes MRP and mdr1 and their products in VP-16 (etoposide)-selected H69 small cell lung cancer cells. *Cancer Res* 1995; **55**:459–462.
29 Matsumoto T, Takano H, Fojo T. Cellular adaptation to drug exposure: evolution of the drug-resistant phenotype. *Cancer Res* 1997; **57**:5086–5092.
30 Modrak DE, Draper MP, Levy SB. Emergence of different mechanisms of resistance in the evolution of multidrug resistance in murine erythroleukemia cell lines. *Biochem Pharmacol* 1997; **54**:1297–1306.
31 Withoff S, de Vries EG, Keith WN, Nienhuis EF, van der Graff WTA, Uges DRA, *et al.* Differential expression of DNA topoisomerase II α and β in P-gp and MRP-negative VM26, mAMSA and mitoxantrone-resistant sublines of the human cell line GLC_4. *Br J Cancer* 1996; **74**:1869–1876.
32 Eijdems EW, de Haas M, Timmerman AJ, Van der Schans GP, Kamst E, de Nooij J, *et al.* Reduced topoisomerase II activity in multidrug-resistant human non-small cell lung cancer cell lines. *Br J Cancer* 1995; **71**:40–47.
33 Moritaka T, Kiura K, Ueoka H, Tabata M, Segawa Y, Shibayama T, *et al.* Cisplatin-resistant human small cell lung cancer cell line shows collateral sensitivity to vinca alkaloids. *Anticancer Res* 1998; **18**:927–934.
34 Bergman AM, Munch-Petersen B, Jensen PB, Sehested M, Veerman G, Voorn DA, *et al.* Collateral sensitivity to gemcitabine (2′,2′-difluoro-deoxycytidine) and cytosine arabinoside of daunorubicin- and VM-26-resistant variants of human small cell lung cancer cell lines. *Biochem Pharmacol* 2001; **61**:1401–1408.
35 Jensen PB, Holm B, Sorensen M, Christensen IJ, Sehested M. *In vitro* cross-resistance and collateral sensitivity in seven resistant small-cell lung cancer cell lines: preclinical identification of suitable drug partners to taxotere, taxol, topotecan and gemcitabin. *Br J Cancer* 1997; **75**:869–877.
36 Mote PA, Davey MW, Davey RA, Oliver L. Paclitaxel sensitizes multidrug resistant cells to radiation. *Anticancer Drugs* 1996; **7**:182–188.
37 Yalowich JC. Effect of microtubule inhibitors on etoposide accumulation and DNA damage in human K562 cells *in vitro*. *Cancer Res* 1987; **47**:1010–1015.
38 D'Incalci M, Schuller J, Colombo T, Zucchetti M, Riva A. Taxoids in combination with anthracyclines and other agents: pharmacokinetic considerations. *Semin Oncol* 1998; **25**:16–20.

39 Yamamoto K, Kikuchi Y, Kudoh K, Nagata I. Modulation of cisplatin sensitivity by taxol in cisplatin-sensitive and -resistant human ovarian carcinoma cell lines. *J Cancer Res Clin Oncol* 2000; **126**:168–172.
40 Christen R, Jekunen A, Jones J, Thiebaut F, Shalinsky D, Howell S. *In vitro* modulation of cisplatin accumulation in human ovarian carcinoma cells by pharmacologic alteration of microtubules. *J Clin Invest* 1993; **92**:431–440.
41 Parker RJ, Dabholkar MD, Lee KB, Bostick-Bruton F, Reed E. Taxol effect on cisplatin sensitivity and cisplatin cellular accumulation in human ovarian cancer cells. *J Natl Cancer Inst Monogr* 1993; **15**:83–88.
42 Rowinsky EK, Citardi MJ, Noe DA, Donehower RC. Sequence-dependent cytotoxic effects due to combinations of cisplatin and the antimicrotubule agents taxol and vincristine. *J Cancer Res Clin Oncol* 1993; **119**:727–733.
43 Judson PL, Watson JM, Gehrig PA, Fowler WC, Haskill JS. Cisplatin inhibits paclitaxel-induced apoptosis in cisplatin-resistant ovarian cancer cell lines: possible explanation for failure of combination therapy. *Cancer Res* 1999; **59**:2425–2432.