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## Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells

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**Abstract** Cisplatin produces good responses in solid tumours including small cell lung cancer (SCLC) but this is limited by the development of resistance. Oxaliplatin is reported to show activity against some cisplatin-resistant cancers but there is little known about oxaliplatin in SCLC and there are no reports of oxaliplatin resistant SCLC cell lines. Studies of drug resistance mainly focus on the cellular resistance mechanisms rather than how the cells develop resistance. This study examines the development of cisplatin and oxaliplatin resistance in H69 human SCLC cells in response to repeated treatment with clinically relevant doses of cisplatin or oxaliplatin for either 4 days or 2 h. Treatments with 200 ng/ml cisplatin or 400 ng/ml oxaliplatin for 4 days produced sublines (H69CIS200 and H69OX400, respectively) that showed low level (approximately two-fold) resistance after eight treatments. Treatments with 1,000 ng/ml cisplatin or 2,000 ng/ml oxaliplatin for 2 h also produced sublines, however, these were not stably resistant suggesting shorter treatment pulses of drug may be more effective. Cells survived the first five treatments without any increase in resistance, by arresting their growth for a period and then regrowing. The period of growth arrest was reduced after the sixth treatment and the H69CIS200 and H69OX400 sublines showed a reduced growth arrest in response to cisplatin and oxaliplatin treatment suggesting that ‘regrowth resistance’ initially protected against drug treatment and this was further upregulated and became part of the

resistance phenotype of these sublines. Oxaliplatin dose escalation produced more surviving sublines than cisplatin dose escalation but neither set of sublines were associated with increased resistance as determined by 5-day cytotoxicity assays, also suggesting the involvement of regrowth resistance. The resistant sublines showed no change in platinum accumulation or glutathione levels even though the H69OX400 subline was more sensitive to buthionine sulfoximine treatment. The H69CIS200 cells were cross-resistant to oxaliplatin demonstrating that oxaliplatin does not have activity against low level cisplatin resistance. Relative to the H69 cells, the H69CIS200 and H69OX400 sublines were more sensitive to paclitaxel and taxotere suggesting that the taxanes may be useful in the treatment of platinum-resistant SCLC. These novel cellular models of cisplatin and oxaliplatin resistant SCLC will be useful in developing strategies to treat platinum-resistant SCLC.

**Keywords** Oxaliplatin · Cisplatin · Drug resistance · Regrowth resistance · Small cell lung cancer · Cellular response

### Introduction

Cisplatin is a widely used chemotherapeutic drug in the treatment of solid tumours including small cell lung cancer (SCLC) [1]. The development of drug resistance is the primary reason for cisplatin’s failure to cure cancer. Over the last 30 years many other platinum drugs have been developed in an attempt to improve on cisplatin. One of these newer drugs is oxaliplatin which has activity against colon cancer in vitro [2] and is now used as a treatment for colon cancer in combination with 5-fluorouracil [3, 4]. Oxaliplatin is also thought to be a better tolerated chemotherapeutic than cisplatin although cases of oxaliplatin toxicity have been reported [5, 6].

Oxaliplatin is widely regarded as useful for the treatment of cisplatin-resistant cancer. Evidence for this

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comes from studies of cisplatin-resistant cell cultures and clinical studies. There is also the notion that because oxaliplatin has a different activity profile to cisplatin in the National Cancer Institute's panel of 60 cell lines [7], oxaliplatin should complement cisplatin treatment and be effective against cisplatin resistance [2]. However, the evidence from cellular studies involve high levels of resistance (20- to 40-fold) to cisplatin [8, 9]. While these highly resistant models are useful to understand the possible mechanisms of resistance, drug resistance in the clinical setting typically occurs at levels of two- to three-fold [10, 11] and may therefore involve different mechanisms of resistance.

The clinical evidence that oxaliplatin is active against cisplatin-resistant cancers involves reports of oxaliplatin having greater activity against platinum pre-treated testicular cancer when combined with other chemotherapeutics such as gemcitabine [12, 13] or irinotecan [14] rather than oxaliplatin as a single agent [15]. In these studies, it is difficult to determine whether it is the oxaliplatin or the combination of drugs that produces a response in cisplatin pre-treated patients. This question is unlikely to be resolved by further clinical trials. The development of clinically relevant cellular models of cisplatin and oxaliplatin resistance would therefore help to resolve this issue. The way platinum resistance is defined in the clinic is also a complicating factor. Platinum pre-treated patients and clinical platinum resistance are not necessarily the same and different criteria are used in different clinical trials. When oxaliplatin was studied as a single agent in ovarian carcinoma where patients were divided into platinum resistant or platinum sensitive based on Markman's criteria [16], there was a clear drop in response rate to oxaliplatin in the cisplatin-resistant patients [17–19]. This suggests that oxaliplatin's activity is reduced in cisplatin-resistant cancer as this cohort failed to respond to oxaliplatin as a single agent.

There are a variety of cisplatin-resistant SCLC sublines that have been established with various cisplatin treatment regimens and 11 examples are presented in

Table 1. The majority of these studies focused on mechanisms of resistance and there was little information on how resistance developed or whether their sensitivity to oxaliplatin was altered. Seven of these sublines were produced by continuous cisplatin treatment for periods longer than a week and had cisplatin resistance from 5- to 25-fold. The other four sublines were repeatedly treated with cisplatin for 1 h to 4 days and gave resistances of 2- to 16-fold. We chose to use pulsed rather than continuous drug exposure for our study of the development of resistance as this may result in low level and possibly more clinically relevant, resistance. Treatment doses were chosen in the range of  $IC_{10}$ – $IC_{40}$  and are consistent with doses used in the clinical setting. Pharmacokinetic studies show that plasma platinum levels peak at a range of 1–10  $\mu\text{g/ml}$  in 2 h with a rapid drop to the  $\text{ng/ml}$  range and then a slow decrease over the next 48 h [20, 21]. Our two time and dose strategies reflect these differing pharmacokinetic phases of the administration of platinum drugs; 2-h treatments at 1,000–8,000  $\text{ng/ml}$  and 4-day treatments at 200–1,600  $\text{ng/ml}$ .

There appear to be no reports of SCLC cells selected for oxaliplatin resistance. We report here the results of a comparative study of cisplatin and oxaliplatin and their ability to induce resistance in the human H69 SCLC cell line when administered repeatedly as either a 4-day or 2-h pulse. The resistant cells produced were then maintained in drug-free media and their mechanisms of resistance and patterns of cross-resistance between cisplatin, oxaliplatin and several other drugs were determined.

## Methods

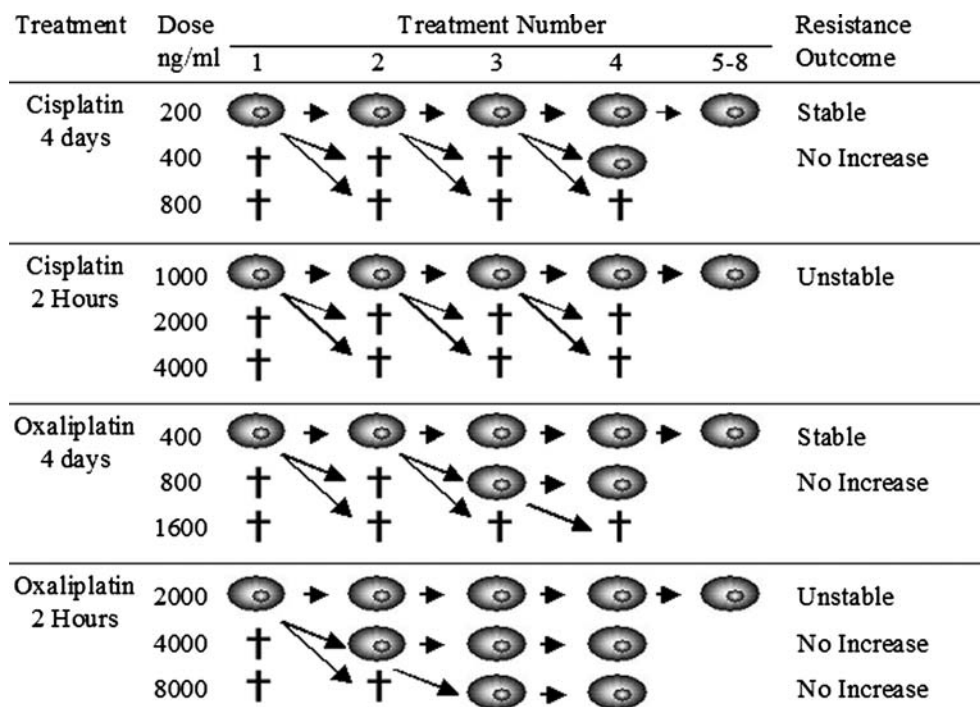
### Cell culture and resistant subline development

The human H69 SCLC cell line was obtained from the American Type Culture Collection. All cells and sublines were maintained in drug-free RPMI (Thermo-

**Table 1** Examples of cisplatin-selected SCLC sublines

Subline (fold resistance)	Treatment	References
H69/0.2 (8)	Continuous 50 $\text{ng/ml}$ for 1–3 weeks. Escalate dose to 200 $\text{ng/ml}$	[26]
H69/0.4 (25)	Same as for H69/0.2 then grown in 400 $\text{ng/ml}$ for 1 year	
N231/0.2 (8)	Continuous 50 $\text{ng/ml}$ for 1–3 weeks. Escalate dose to 200 $\text{ng/ml}$	
H69/CPR (5)	Continuous exposure in escalating doses up to 400 $\text{ng/ml}$ over 4–6 months	[27]
H69-CP (3)	Six treatments of 100 $\text{ng/ml}$ for 4 days with 2–3 weeks recovery between	[28]
H82-CP (2)	Six treatments of 100 $\text{ng/ml}$ for 4 days with 2–3 weeks recovery between	
GLC <sub>4</sub> -CDDP (6)	Continuous exposure with more drugs added each time the cells grew. Nine such treatments over 1 year. Maintained by 1 h exposure per month	[29]
SBC-3/CDDP (13)	Continuous starting at 30 $\text{ng/ml}$ for 2–3 weeks then escalating up to 1,500 $\text{ng/ml}$ over 2 years	[30]
H209/CP (11.5)	Continuous 80 $\text{ng/ml}$ for several months then maintained by treating every 2 weeks	[31]
SW2/CDDP (3.3)	$IC_{50}$ dose for 1 h per week with dose escalation of 15–20% per treatment when possible over 14 months	[32]
(SCLC1)SR-2 (16)	Five nanograms per millilitre for 24 h per 3 weeks; maintained with 100 $\text{ng/ml}$	[33]

**Fig. 1** Drug treatment regimens and the development of resistance. The H69 SCLC cell line was treated with 12 different regimens as indicated. Cultures surviving a treatment are represented by a *cell image* while unsuccessful treatments are represented by a *cross*



electron, Sydney, Australia) with 10% FCS (Thermo-electron) in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. The H69 cells were treated as shown in Fig. 1 with cisplatin for 4 days at doses of 200, 400 or 800 ng/ml; with cisplatin for 2 h at doses of 1,000, 2,000 or 4,000 ng/ml; with oxaliplatin for 4 days at doses of 400, 800 or 1,600 ng/ml or with oxaliplatin for 2 h at doses of 2,000, 4,000 or 8,000 ng/ml. Following treatment cells were transferred to drug-free culture conditions for recovery and when cultures had undergone approximately five doublings, the treatment was repeated either at the same drug dose or at higher doses as indicated in Fig. 1. Eight consecutive treatment cycles were performed on cultures over an 8-month period. All cultures were *mycoplasma* free.

#### Cytotoxicity assay

To determine the level of resistance, cells were plated into flat bottomed 96-well plates at a cell density of  $6.0 \times 10^4$  cells/well. Cells were treated in triplicate with two-fold serial dilutions of drug in a final volume of 200 µl. Drug-free controls were included in each assay. Plates were incubated for 5 days at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and cell viability was determined using the MTT assay [22]. Fifty microlitres of MTT (2.5 mg/ml in PBS) was added to each well and the cells incubated for a further 2 h. The plates were centrifuged at 800g for 5 min, the culture medium aspirated and the formazan product dissolved in 100 µl DMSO. Plates were mixed for 15 min and the absorbance measured at 570 nm. Cell viability was calculated as a percentage of control absorbance values and the fold

resistance was calculated by dividing the IC<sub>50</sub> of the resistant cells by that of the H69 cells.

#### Glutathione assay

Total intracellular glutathione was determined using a modification of the colorimetric method of Suzukake et al. [23] as previously described [24].

#### Flow cytometry cell cycle analysis

Cells ( $10^6$ ) were resuspended in 500 µl of Dulbecco's PBS containing 50 µg/ml propidium iodide and 0.02% nonidet P-40 on ice. The cells were then incubated on ice for 10–15 min and analysed in a Becton Dickinson FACScan flow cytometer. Red fluorescence was monitored (FL2) using a 585/42 band pass filter, 10,000 events were collected and the data was analysed using CellQuest software.

#### Platinum accumulation

Cells ( $2.5 \times 10^6$ ) were washed in 10 ml PBS, centrifuged and the supernatant carefully removed. The pellet of platinum treated cells was dried on a heating block, resuspended in 100 µl of nitric acid and incubated at 90°C for 3 h. Samples were then resuspended in 200 µl of 0.1 M HCl and analysed by Atomic Absorption using a Platinum Photron hollow cathode lamp in a Varian SpectrAA-400-Zeeman spectrophotometer using the operating conditions as specified by the manufacturer.

## Statistical analysis

The Student's *t*-test was used to determine significant differences. A *P*-value of less than 0.05 was regarded to be significant.

## Results

### Development of platinum resistance

The H69 cells were treated as shown in Fig. 1 and as described in Section 'Methods'. Of the 12 different initial treatments, only the lowest drug concentrations produced surviving cells. These lowest drug concentration treatments all produced between 20 and 30% cell death and growth arrest. On drug treatment, cells increased in size and did not aggregate in typical SCLC clumping morphology. Surviving cultures were then re-treated when their normal growth rate and clumping morphology had returned. Cultures were treated with the same drug and dose as well as with twice and four times that dose. All cultures again survived the lowest dose but none survived the higher doses except for those treated with 4,000 ng/ml oxaliplatin for 2 h. The results of the third treatment with the same and with escalated doses produced cultures that survived the same dose, none of the cultures treated with increased cisplatin doses survived while increased oxaliplatin produced two surviving cultures (Fig. 1). Subsequent treatments with increased doses of cisplatin were performed out to treatment 4 which produced one culture that survived treatment with 400 ng/ml cisplatin for 4 days. Cultures therefore appeared to survive oxaliplatin dose escalation more easily than cisplatin dose escalation. However, those cultures surviving dose escalation were not more resistant than the cultures from which they were derived as determined by the standard 5-day cytotoxicity assay suggesting that this resistance may be associated with growth delay.

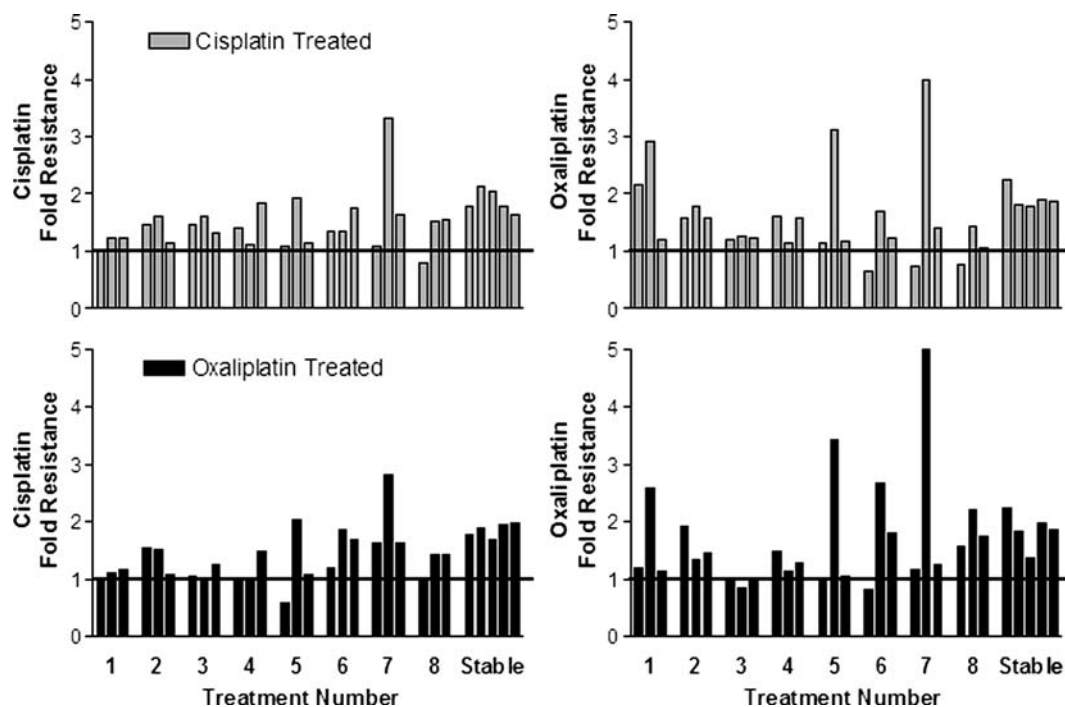
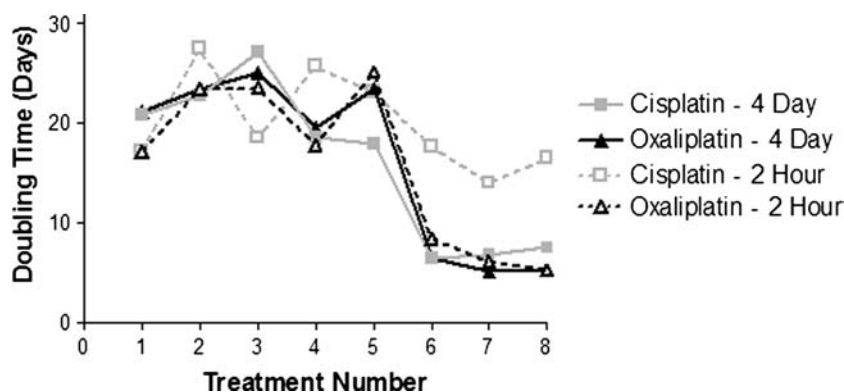
Dose escalation of platinum drugs rarely occurs as part of SCLC treatment [25]. We therefore concentrated on characterising the development of resistance in the cultures repeatedly treated with the same lowest dose schedule. The initial treatments with cisplatin and oxaliplatin for 4 days produced a growth arrest and a time to doubling of 21 days while the 2 h treatments resulted in a 17-day recovery (Fig. 2). A similar growth arrest occurred in all schedules for the first five treatments. For the sixth, seventh and eighth treatments, the recovery period was reduced to 6 days in all except the 2-h cisplatin schedule. The cell sublines resulting from eight treatments of the 4-day cisplatin schedule were designated H69CIS200; the 2-h cisplatin schedule, H69CIS1000; the 4-day oxaliplatin schedule, H69OX400; the 2-h oxaliplatin schedule, H69OX2000. The resistant cells were of the same size and morphology as the parental cells and grew at a similar growth rate in drug-free media (data not shown).

The level of resistance to cisplatin and oxaliplatin was monitored after each treatment at weekly intervals after recovery by performing a 5-day cytotoxicity assay. The results in Fig. 3 show that for the 4-day cisplatin and oxaliplatin schedules, low level (approximately two-fold), stable resistance developed following the eighth treatment. A similar level and pattern of stable cross-resistance to the non-selecting platinum drug was also evident. Although the 2 h cisplatin and oxaliplatin schedules showed similar trends to those of the 4-day schedules (not shown), they did not produce stable resistance after the eighth treatment and therefore they were not included in further studies. Resistance to oxaliplatin was detected earlier than resistance to cisplatin in both sublines. A higher level of oxaliplatin resistance was also detected in comparison to cisplatin resistance in both resistant sublines. Resistance appeared to be greatest in the second week after recovery. However, this resistance was transient as the level of resistance was usually lower in the third week. This variation was largest for the oxaliplatin treatments as compared to the cisplatin treatments and it was most evident at treatment 7. This increased variation may be related to the drop in doubling time at treatment 6 but may not be part of the progression to stable resistance as it did not re-occur at treatment 8.

### Changes associated with stable low-level resistance

The effect of an acute drug treatment on recovery time was determined by counting cells microscopically following treatment of the H69 cells and the H69CIS200 subline with 1,000 ng/ml cisplatin for 2 h and the H69 cells and the H69OX400 subline with 2,000 ng/ml oxaliplatin for 2 h. Figure 4 shows that the doubling time after cisplatin treatment for the H69CIS200 subline was 10 days compared to 18 days for the H69 cells. The doubling time after oxaliplatin treatment was even shorter for the H69OX400 subline (5 days) compared to greater than 21 days for the H69 cells. These shorter doubling times were reflected in the time for the cell cycle to return to normal (Fig. 5). When the H69 and H69CIS200 cells were treated with 1,000 ng/ml cisplatin for 2 h; the time for the recovery of the sub-G<sub>0</sub> phase from 25% to a normal 4% and for the return of the G<sub>0</sub>/G<sub>1</sub> phase to a normal 60% was faster for the H69CIS200 cells than the H69 cells. There was also an increase in the proportion of H69 cells in the G<sub>2</sub>/M phase, there was no such change for the H69CIS200 subline. The H69OX400 subline treated with 2,000 ng/ml oxaliplatin for 2 h showed little change in the cell cycle relative to those changes seen in the H69 cells. Even though 1,000 ng/ml cisplatin and 2,000 ng/ml oxaliplatin produced a more dramatic change in cell cycle in the H69 cells than in both the resistant sublines, when the H69CIS200 subline was acutely treated with double the dose (2,000 ng/ml cisplatin for 2 h) and the H69OX400 subline with four times the dose

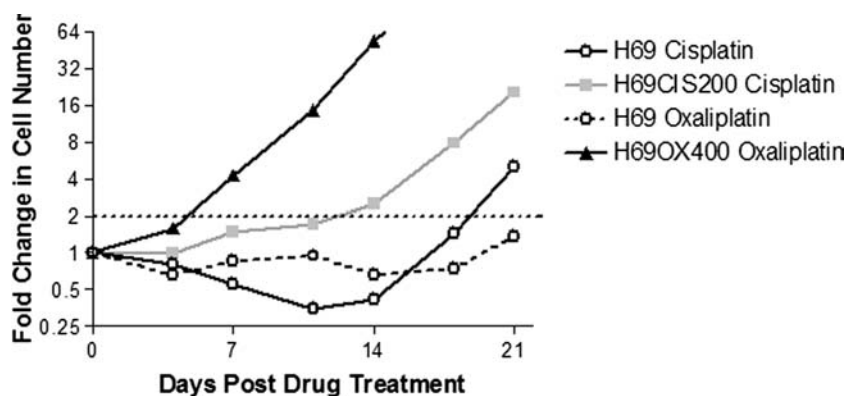
**Fig. 2** The recovery time following each treatment. The number of cells that exclude trypan blue were counted twice a week following treatment and the time taken to double cell number was determined



**Fig. 3** Resistance to cisplatin and oxaliplatin following each treatment. The resistance to cisplatin and to oxaliplatin was determined for three consecutive weeks following recovery from

each treatment using a 5-day cytotoxicity assay in which viability was determined by the MTT assay. After eight treatments, the resistance was monitored weekly for 5 weeks

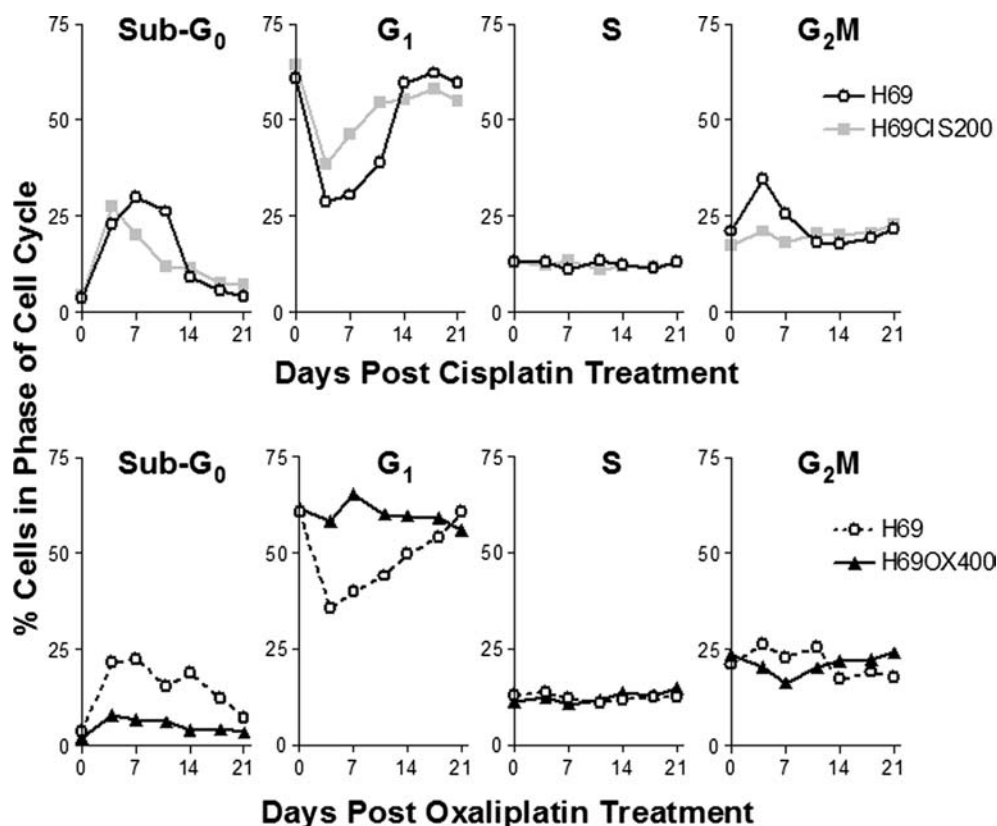
**Fig. 4** Effect of acute drug treatment on cell growth. The H69CIS200 and H69 cells were treated with 1,000 ng/ml cisplatin for 2 h and the H69OX400 and H69 cells were treated with 2,000 ng/ml oxaliplatin for 2 h. The number of cells that exclude trypan blue were counted and the fold change was plotted vs time after treatment



(8,000 ng/ml oxaliplatin for 2 h), the cell cycle profiles of the sublines resembled those for the H69 cells (not shown). The cell cycle kinetics were also determined

during the development of the sublines following treatment cycle 4 and found to be the same as for the treated H69 cells (not shown).

**Fig. 5** Effect of acute drug treatment on cell cycle. Cells were treated as described in Fig. 4 and the proportion of cells in each phase of the cell cycle was determined by the propidium iodide/flow cytometry method described in Section 'Methods'



### Cross-resistance

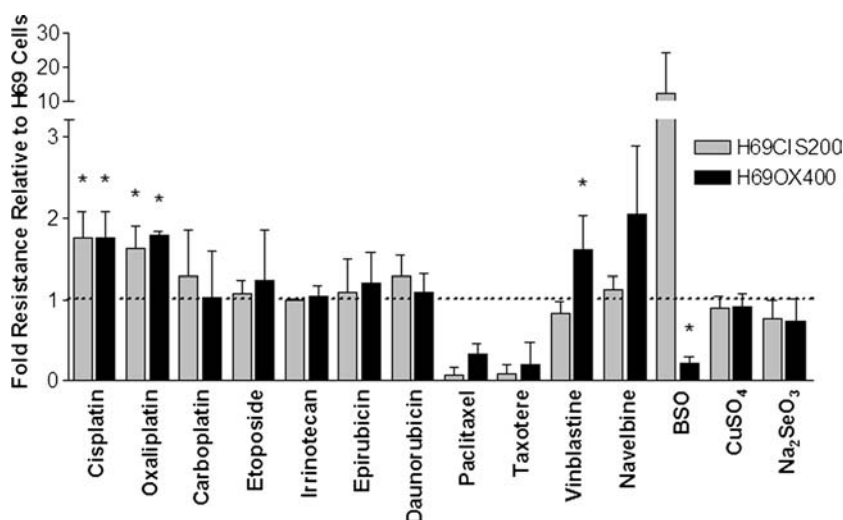
The H69CIS200 and H69OX400 cell sublines were equally resistant to cisplatin and oxaliplatin but they were not significantly resistant to carboplatin (Fig. 6). Neither subline showed resistance to daunorubicin, epirubicin, etoposide, selenium or copper. However, both the resistant sublines showed increased sensitivity to paclitaxel and taxotere. This increase in sensitivity was not associated with other mitotic spindle poisons

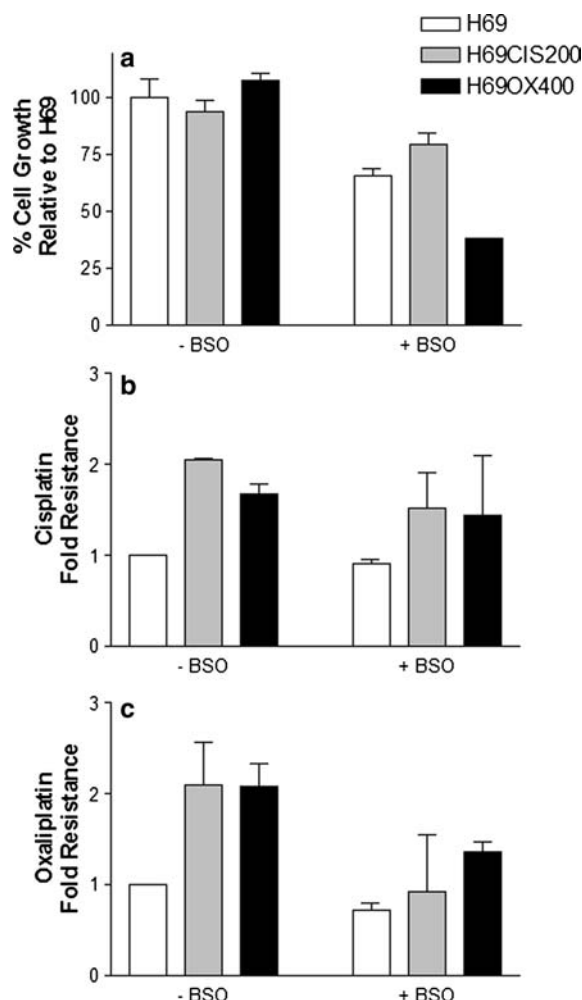
such as vinblastine or navelbine. Rather the H69OX400 subline was resistant to vinblastine. The H69CIS200 subline was resistant to buthionine sulfoximine (BSO) while the H69OX400 subline was sensitive to BSO.

### Cellular glutathione and resistance

To further investigate this differential effect of BSO, the levels of cellular glutathione was determined

**Fig. 6** Cross-resistance of the H69 sublines. The cross-resistance of the H69CIS200 and H69OX400 sublines to the indicated drugs was determined using a 5-day cytotoxicity assay. The mean fold resistance relative to the H69 cells is plotted





**Fig. 7** Effect of glutathione depletion on cell growth and drug resistance. **a** The number of cells which exclude trypan blue was determined after 3 days in culture  $\pm$  50  $\mu$ M BSO. The  $IC_{50}$  for **b** cisplatin and **c** oxaliplatin was determined for a 5-day cytotoxicity assay in the presence and absence of 50  $\mu$ M BSO and the fold resistance calculated relative to the H69 cells in the absence of BSO. The means and standard deviations of two separate experiments are shown

following 24 h culture in fresh media. There was no significant difference in glutathione levels between the sublines and platinum drug treatment had no effect on glutathione levels (not shown). Fifty micromolar BSO depleted glutathione in all the cell lines to a similar extent of approximately 2% of the untreated level (not shown).

The effect of depleting cellular glutathione on cell growth and on resistance was determined by culturing the H69 cells and the resistant sublines in media containing 50  $\mu$ M BSO. Figure 7a shows that BSO treatment reduced the growth of the H69 cells to 65% of untreated cells, for the H69CIS200 subline the reduction was similar but for the H69OX400 subline growth was further inhibited. BSO tended to sensitise all cells to oxaliplatin, but had little effect on cisplatin resistance (Fig. 7b, c).

## Platinum accumulation

There were no significant changes in the level of cell-associated platinum in the H69CIS200 or H69OX400 sublines relative to the H69 cells following a 2 h exposure to 1,000 ng/ml cisplatin, 2,000 ng/ml oxaliplatin or a 4-day exposure to 200 ng/ml cisplatin or 400 ng/ml oxaliplatin (not shown). This suggests that changes in drug efflux or drug uptake were not contributing to resistance.

## Discussion

Studies of cellular drug resistance mainly focus on the molecular mechanisms contributing to the resistance rather than how the cells became resistant and the factors that promote its development. In this study, the H69 SCLC cells were treated either for 4 days or 2 h with an  $IC_{20}$  dose of cisplatin or oxaliplatin to determine the impact of cisplatin vs oxaliplatin and the length of drug exposure on the development of resistance. The time taken to develop stable resistance was approximately 8 months and this compares with the range of 4–24 months reported for other cisplatin-resistant SCLC sublines [26–33]. The rate of development of resistance appeared similar between the 2-h and 4-day treatment schedules. However, the 4-day schedule produced stable resistance while the 2-h schedule produced unstable resistance suggesting that a shorter pulse may be more effective against cancer than continuous exposure or longer pulse times. This is similar to what was observed in the development of a cisplatin-resistant ovarian carcinoma, which yielded stable resistance from a continuous exposure but not from a series of 1-h pulses [34]. However, other studies have produced stable cisplatin resistance with 1–2 h pulses in murine ovarian reticulosarcoma [35], human ovarian adenocarcinoma [36] and non-SCLC [37] using higher doses of drug ( $\mu$ M) than used in this study.

Our results show that SCLC cells develop resistance to oxaliplatin more easily than they develop resistance to cisplatin. While we could find no other reports of oxaliplatin selected SCLC cells, there are many reports for other types of cell lines. Commonly reported mechanisms of resistance include increased cellular glutathione [38] and decreased platinum accumulation [39–41] similar to what is seen in cisplatin resistance. The H69CIS200 and H69OX400 sublines appear to have neither decreased platinum accumulation nor increased glutathione but rather may rely on altered cell cycle kinetics as a means of resistance (Figs. 4, 5).

During the first five treatment cycles, there was no measurable drug resistance yet the treated cells survived and regrew. Survival of these early treatment cycles involved a reversible cell cycle arrest followed by regrowth. Regrowth resistance has previously been reported as a mechanism that allows cells to survive drug treatment via proliferation rather than increased drug

resistance [42]. Depending on the rate of proliferation and the sensitivity of the malignancy to therapy, the effects of tumour regrowth can range from insignificant to the complete offsetting of the effects of treatment [43]. After six treatment cycles, the response of the resistant cells changed. This is comparable to the resistance observed after six treatment cycles in cisplatin-resistant SCLC [28] and six treatment cycles in oxaliplatin-resistant ovarian carcinoma [39].

There was a shorter time to doubling for three out of the four resistant sublines and this change in response was accompanied by a change in the cell cycle kinetics following drug treatment (Fig. 5). Earlier in the development of resistance (treatment cycle 4) treated cells showed a similar cell cycle recovery as the H69 cells. A similar pattern of growth arrest and recovery was observed in the development of cisplatin-resistant IGROV1 ovarian carcinoma cells [36]. IGROV1 cells were exposed to cisplatin for 2 h and allowed to recover for several weeks. Development of resistance to cisplatin was associated with the ability of the treated cells to progress through the cell cycle beyond the G1/S checkpoint; although most cells died by apoptosis, a few surviving cells proliferated and recolonised the cultures. The authors suggested that this was not resistance to drug induced cell death, rather an increased propensity to proliferate after cytotoxic treatment [36], in other words regrowth resistance. It is likely that regrowth resistance is initially used as a survival mechanism that also provides the time for a more permanent protective mechanism to develop.

Regrowth resistance is difficult to measure using a conventional 5-day cytotoxicity assay. The main reason for this is that regrowth does not occur within the time of the assay. Also, the cytotoxicity assay depends on their being a change in growth rate or survival with changing dose of test drug. For regrowth resistance, growth arrest can occur over a wide drug concentration range effectively producing no change in growth or survival for the cytotoxicity assay to detect. Our sublines which survived dose escalation showed no increase in resistance in a conventional 5-day assay, however, their survival is indicative of regrowth resistance.

As to whether oxaliplatin is more effective than cisplatin, there is little in the way of direct comparisons in cellular resistance studies. Our results show resistance developed in a similar manner in response to cisplatin and oxaliplatin in our SCLC cell model. Both produced similar levels of resistance (Fig. 3), drug cross-resistance (Fig. 6) and stability of the resistance (Fig. 3). However, it was easier to escalate the dose of oxaliplatin compared to cisplatin. A two-fold higher dose of cisplatin was cytotoxic to low-level resistant cells while a two-fold higher dose of oxaliplatin still resulted in viable cells. This suggests oxaliplatin may be less effective than cisplatin. This is also supported by the quicker recovery of growth from a single drug treatment for the H69OX400 subline compared to the H69CIS200 subline (Fig. 4). A possible explanation for the faster recovery from oxaliplatin treatment and the greater number of oxaliplatin

surviving sublines, is the greater efficiency of bypassing of oxaliplatin-DNA adducts than cisplatin-DNA adducts by DNA polymerases [44]. There is also further evidence to suggest that at equimolar concentrations oxaliplatin forms fewer but more cytotoxic DNA lesions than cisplatin [45, 46]. This may explain the response of our sublines to equally cytotoxic doses of drug, in the case of oxaliplatin there may be fewer lesions with a better chance of being bypassed by DNA polymerases. This combination leads to a greater chance of the oxaliplatin treated cell dividing, despite the presence of DNA lesions. The cell division will dilute out the number of lesions per cell and these surviving cells are likely to have additional attributes contributing to their mechanism of resistance to the platinum drug.

There is evidence to suggest that oxaliplatin is active against cisplatin-resistant cancers and cells [8, 9, 12–14]. This has also been reported in SCLC, in one study the 16-fold cisplatin-resistant SR2 SCLC subline was sensitive to oxaliplatin [33] while another variant of this subline that was 3.3-fold resistant to cisplatin, was 1.4-fold resistant to oxaliplatin [47]. In our study of the H69 SCLC cells, oxaliplatin did not have activity against the cisplatin-resistant H69CIS200 cells and there are similar reports in ovarian carcinoma [40]. This also complements the clinical studies showing a lack of activity of oxaliplatin in cisplatin-resistant ovarian carcinoma [17–19]. Our study questions the effectiveness of oxaliplatin in cisplatin-resistant cancer and suggests that more research into the mechanisms of low-level platinum resistance is needed to resolve this issue.

Even though oxaliplatin had little activity against cisplatin resistance in our study, paclitaxel and taxotere showed increased activity against both the H69CIS200 and H69OX400 sublines relative to the H69 cells (Fig. 6). There are previous reports of cisplatin-resistant SCLC cells being sensitised by pre-treatment with a low dose of paclitaxel [28] and there are many examples of other cisplatin-resistant cell lines that are sensitive to taxanes [48–52]. Taxanes bind to and stabilise microtubules and block cell cycle progression through centrosomal impairment, induction of abnormal spindles and suppression of spindle microtubule dynamics [53]. The mechanism of platinum resistance in these resistant sublines may involve tubulin abnormalities which then render the cells sensitive to subsequent paclitaxel treatment. This is supported by the report of cisplatin resistance being associated with decreased levels of  $\beta$ -tubulin and tubulin abnormalities [48, 54]. Another possible explanation could involve survivin since this is increased in cisplatin-resistant ovarian cancer cells and paclitaxel treatment reduces survivin levels in these cells [55].

Increases in intracellular glutathione has been previously associated with platinum resistance in many studies [10, 38, 56]. We have determined that the level of glutathione remains unchanged between the cell lines and treatment with 50  $\mu$ M BSO tended to sensitise all cells to oxaliplatin, but had little effect on cisplatin resistance (Fig. 7b, c). This suggests that a depletion of

glutathione is not enough to overcome the platinum resistance in this model. All the cell lines show a drop in growth rate in response to 50  $\mu$ M BSO (Fig. 7a), however, the H69OX400 cells show the most inhibition and the H69CIS200 cells grow more than the parental cells under these conditions. This corresponds to what was found in the BSO toxicity assay, the H69CIS200 cells are resistant and the H69OX400 cells are sensitive to BSO (Fig. 6). How this difference in response to BSO treatment relates to platinum resistance will be examined further in this cell model.

We have shown that both cisplatin and oxaliplatin treatment cause the development of resistance in the H69 SCLC cell line in similar ways that initially involves growth arrest–regrowth resistance followed by more permanent resistance mechanisms that appear to involve neither decreased platinum accumulation nor increased glutathione levels. Oxaliplatin was not effective against this cisplatin resistance, however, both resistant sublines were more sensitive to paclitaxel and taxotere suggesting the taxanes should be further investigated for their potential against platinum-resistant SCLC.

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