Eur J Cancer Clin Oncol, Vol. 25, No. 4, pp. 619–625, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Characterization of Cisplatin-resistant COLO 316 Human Ovarian Carcinoma Cells

PAUL A. ANDREWS, MICHAEL P. MURPHY and STEPHEN B. HOWELL
Cancer Center T-012, University of California, San Diego, La Jolla, CA 92093, U.S.A.

Abstract—*The biochemical changes responsible for acquired resistance to cisplatin (DDP) are not fully understood. We have developed DDP-resistant sublines of COLO 316 human ovarian carcinoma cells in vitro and characterized a number of biochemical features of these cells. Following selection with either continuous 50 nM DDP (COLO/DDP$_{50}$ cells) or intermittent 1 μM DDP (COLO/B, COLO/C, or COLO/D cells) the onset of resistance was rapid. The resistance of the COLO/B cells gradually fell from 14-fold to 5-fold over 6 months in drug-free media. Both selection procedures produced cells exhibiting broad cross-resistance to other platinum analogs, natural products and alkylating agents. There was no significant change in the growth rate (doubling time = 36 h, cloning efficiency (28%), protein content (0.55 mg/10^6 cells), or morphology of these cells. Cell cycle distributions of log-phase cells were similar (60% G_0/G_1, 35% S, 5% G_2/M) as determined by flow cytometry. Glutathione (GSH) levels, while not elevated in COLO-B cells at low levels of resistance (2–3-fold), were 30% elevated at higher levels of resistance (9-fold). However, GSH levels in COLO/DDP$_{50}$ cells with 13-fold resistance were 2.3-fold elevated. The resistance of both cell types could be partially reversed by extended depletion of GSH with D,L-buthionine-S,R-sulfoximine. COLO/D cells had a 48% decrease in DDP accumulation at 1 h while COLO/DDP$_{50}$ cells had no change in DDP accumulation. The cross-resistance profiles, GSH biochemistry and DDP accumulation data indicate that acquired DDP-resistance is a complex, multifactorial response in these cells. The specific combination of mechanisms expressed in these cells appears to depend upon the selection procedure.*

INTRODUCTION

CISPLATIN (DDP) is an important drug for the treatment of human ovarian, testicular, bladder and head and neck cancers [1]. Despite the good response rates of these tumors to DDP, the emergence of DDP resistance is a common consequence of therapy [2–4]. Acquired resistance to DDP thus presents a serious barrier to the successful management of these diseases. The rational approach to the prevention, reversal, or circumvention of DDP resistance requires a fundamental understanding of the mechanisms involved. The biochemical and genetic mechanisms of DDP resistance, however, are not yet fully understood.

We have been interested in defining the origins of DDP resistance in human ovarian carcinoma. A number of useful models have been developed in human ovarian carcinoma cells for the study of DDP resistance [5–9]. We have generated DDP-resistant 2008 human ovarian carcinoma cells *in vitro* and found that most of the resistance can be attributed to decreased drug accumulation [5]. DDP-resistant A2780 ovarian carcinoma lines have also been developed by selection *in vitro* [7–9]. These cells have elevated glutathione (GSH), increased unscheduled DNA synthesis and changes in their folate metabolism [7–9]. Their resistance could be reversed by GSH depletion with D,L-buthionine-S,R-sulfoximine (BSO) [7]. DDP-resistant cells (PE04) from a DDP-refractory ovarian carcinoma patient were also recently described and compared to a sensitive line (PE01) established from this same patient prior to chemotherapy [10]. These DDP-resistant cells had elevated GSH and GSH transferase. DDP-resistant KF-1 cells have also been generated, but the possible mechanisms of resistance remain uncharacterized [11]. In this paper we describe the development and characterization of DDP-resistant sublines of COLO 316 human ovarian carcinoma cells.

Accepted 20 October 1988.
Address all correspondence and requests for reprints to: Paul A. Andrews, Cancer Center, UCSD T-012, La Jolla, CA 92093, U.S.A.
Supported by Grant CA-23100 from the National Cancer Institute and a grant from Bristol-Myers Co. This work was conducted in part by the Clayton Foundation for Research, California Division. Drs Andrews and Howell are Clayton Foundation Investigators.

MATERIAL AND METHODS

Drugs and chemicals

DDP, carboplatin, iproplatin, (1,2-diaminocyclohexane)platinum(II) malonate (DACH malonate), *trans*-diamminedichloroplatinum(II) (transDDP), etoposide, melphalan, mitomycin C and adriamycin were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI (Bethesda, MD). Bleomycin was obtained from Bristol-Myers Co. (Syracuse, NY) and 4-hydroperoxycyclophosphamide was obtained from Dr Michael O. Colvin of the Johns Hopkins Oncology Center (Baltimore, MD). 3-*O*-[methyl-^{3}H]D-glucose (79 Ci/mmol) was obtained from NEN Research Products (Boston, MA).

Cell lines

COLO 316 cells established from a patient with serous cystadenocarcinoma of the ovary were used in these studies [12]. Cells were grown as monolayer cultures in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum, 2 mM freshly added glutamine and intermittently with penicillin/streptomycin (Irvine Scientific, Santa Ana, CA). Cultures were equilibrated with humidified 5% CO_2 in air at 37°C. Cells were screened for mycoplasma with a Gen-probe mycoplasma detection kit (Fisher, Tustin, CA).

Two approaches were taken to the development of DDP-resistant sublines in COLO 316 cells: intermittent high dose exposure and continuous low dose exposure. For high-dose exposure, subconfluent cultures were treated with 1 μm DDP in complete medium. After 3 days, the cells were passaged 1:1 and allowed to recover (2–4 weeks). When the cells returned to an actively dividing, healthy state, their sensitivity to DDP was determined by clonogenic assay on plastic, as previously described [13], and they were retreated with DDP. Three derivations were conducted and these cells are designated COLO/B, COLO/C and COLO/D. For low-dose exposure, cells in exponential growth were passaged twice per week in medium containing 50 nM DDP. This is the concentration causing 50% inhibition of colony formation (IC_{50}) for DDP, as determined by clonogenic assay in which the drugs were left in the medium for the full period of colony formation. The sensitivity to DDP for this subline, designated COLO/DDP_{50}, was monitored at routine intervals (every five passages).

Cross-resistance profiles

Sensitivity to DDP and a panel of anticancer agents was determined by continuous exposure clonogenic assay [13]. Resistance was determined by the ratio of the IC_{50}s.

Flow cytometry

Cells were trypsinized with 0.05% trypsin–EDTA and resuspended in 0.9% saline containing 1 mg/ml ribonuclease I (Sigma) and 0.01% Triton X-100. After 1 h, propidium iodide (Calbiochem, San Diego, CA) was added to give a final concentration of 5.0 μg/ml and the cells were analyzed on a Cytofluorograf 50 with a 2151 data handling system (Ortho Diagnostics Systems, Westwood, MA). Stained cells were excited with the laser tuned to 488 nm and an output of 250 mW. Fluorescence was detected through a 630 nm bandpass filter. A cytogram of propidium iodide fluorescence peak vs. area was used to eliminate doublets. Distribution of cells in cell cycle compartments was analyzed by the constant method (Ortho). Forward scatter was determined on intact, unstained cells.

Biochemical methods

Protein was determined by the method of Bradford with bovine serum albumin as standard [14]. GSH was determined as previously described [15]. GSH levels were determined as the average of values obtained over sequential days when cells were in log-phase growth. Cell volumes were determined with 3-*O*-[methyl-^{3}H]D-glucose by the method of Kletzien *et al.* [16].

Chromosome analysis

Karyotypes were performed either by Dr Deborah Saxe at the Agouron Institute (San Diego, CA) or Mr Mark Bogart in the Department of Medicine, University of California, San Diego. Cells were harvested by standard cytogenetic techniques and the chromosomes stained with Giemsa.

Growth rates

Parental and resistant cells were seeded into six-well plates (9.6 cm^2, Costar). Each day wells were trypsinized and cell numbers determined with a Coulter counter.

DDP accumulation

Accumulation of DDP was determined in parent and resistant cells with unlabeled DDP, as described [5]. Cells were seeded into 100 mm tissue culture plates and when the cells approached confluency the medium was replaced with 5 ml RPMI 1640 containing 200 μM DDP. After 1 h incubation, the medium was aspirated, the monolayers washed once with 4°C 0.05% trypsin–EDTA, and then trypsinized with 5 ml of 37°C 0.05% trypsin–EDTA for 6 min. The trypsinized cells were added to 10 ml of 4°C complete medium and centrifuged for 3 min at 500 ***g***. The cell pellet was resuspended in 10 ml 4°C PBS and centrifuged again. The cell pellet was then resuspended in 1.0 ml 4°C 0.9%

saline and sonicated for 30 s at a power setting of 3 and a 30% duty cycle (5 W pulsed) (Sonifier 450, Branson, Danbury, CT). One hundred μl were removed and mixed with 100 μl 1 N NaOH for protein determination. The remaining cell lyzate was then analyzed for Pt by atomic absorption spectroscopy using a Perkin–Elmer 373 atomic absorption spectrophotometer equipped with a 2200 graphite furnace (Perkin–Elmer, Norwalk, CT). The following heating program was used: 90°C for 50 s, ramp to 1300°C in 10 s and hold for 30 s, 2500°C under maximum power for 7 s.

GSH depletion

BSO was added to flasks of cells to give a final concentration of 50 μM. Control flasks received no BSO. After 24 h, cells were trypsinized and diluted to give 80 cells/ml in complete medium. BSO treated cells were diluted into medium that contained 50 μM BSO. Five ml of suspended cells were plated into 60 mm polystyrene tissue culture dishes (Becton Dickinson Labware, Oxnard, CA). Fifty μl of DDP stock solutions were added to triplicate plates at each drug concentration. Control plates received diluent alone. Plates were incubated in 5% CO_2 in air at 37°C. After 24 h the medium was removed in all the plates and replaced with fresh medium containing no drugs or BSO. After an additional 13 days, plates were fixed with methanol and stained with Giemsa. Colonies of over 60 cells were counted macroscopically. Statistical significance of dose modification factors (DMF) were determined by *t*-tests on grouped data.

RESULTS

Generation of DDP resistance

We generated resistant sublines of COLO 316 cells by two separate selection procedures. When cells were selected monthly with 1.0 μM DDP, a concentration that kills six logs of parent cells, the DDP-resistance appears rapidly and steadily increases (Fig. 1). Cells surviving the first selection were already 2–3-fold resistant. Although there was variation in the rate of development of resistance in the three derivations, a general upward trend in the level of resistance was found. However, in two cases, COLO/C and COLO/D cells, and possibly in the COLO/B cells, resistance fell with continued selection. In the case of COLO/B and COLO/C cells we believe these declines may be attributable to mycoplasma contamination (*vide infra*). The maximum resistance obtained was 17-fold. In the absence of DDP selection, the resistance of COLO/B cells gradually declined over the course of 6 months from 14-fold to 5-fold (Fig. 2A). The linear regression line through these points was $y = 0.082x + 18.63$ ($r = 0.77$). Although some drift in the IC_{50} value for COLO 316 cells was noted, the majority of this declining resistance was indeed due to the decreasing IC_{50} values of the COLO/B cells (Fig. 2B). The other approach taken to the development of DDP-resistance was to expose the cells chronically to a concentration of DDP that kills 50% of the cells in a clonogenic assay. Initially there was no change in resistance when the cells were passaged in 50 nM DDP (Fig. 3). After 10–15 passages, however, the resistance began to develop and steadily increased. The highest resistance obtained was 16-fold. Similar to the observations made with intermittent selection, further selection after this high level of resistance was obtained, led to decreases in resistance.

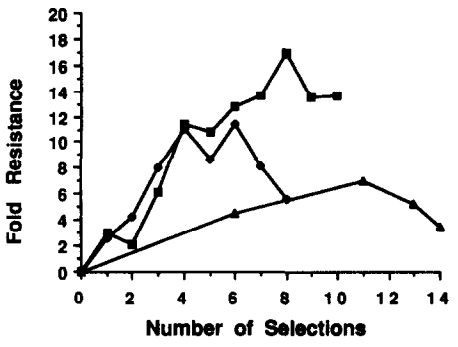

Fig. 1. Development of DDP resistance in COLO 316 cells selected by intermittent high dose exposure. Cells were exposed to 1.0 μM DDP at approximate 1 month intervals. When healthy cultures were re-established, DDP-resistance was determined by clonogenic assay on plastic. Lines represent three separate derivations of resistant cells: (■) COLO/B; (♦) COLO/C; (▲) COLO/D.

Growth characteristics

Both COLO/B and COLO/DDP_{50} cells had doubling times of 36 h which were identical to the parent COLO 316 cells. There was no significant change in the cloning efficiency (28%) or morphology of these cells. Flow cytometry did not reveal any differences in the cell cycle distribution of these cells (Table 1). Forward scatter was also the same in the sensitive and resistant cells indicating that there were no obvious differences in the cell size or cell surface properties of the trypsinized cells. The protein content of all three cell types was 0.55 ± 0.03 mg/10^6 cells.

Karyotypes

Karyotypic analysis confirmed that the resistant cells were of COLO 316 origin [10]. No double minute chromosomes or homogeneously staining regions, indicative of gene amplification, were observed (data not shown). In addition, no gene rearrangements or deletions were identified in the resistant cells that were not present in the parent cells.

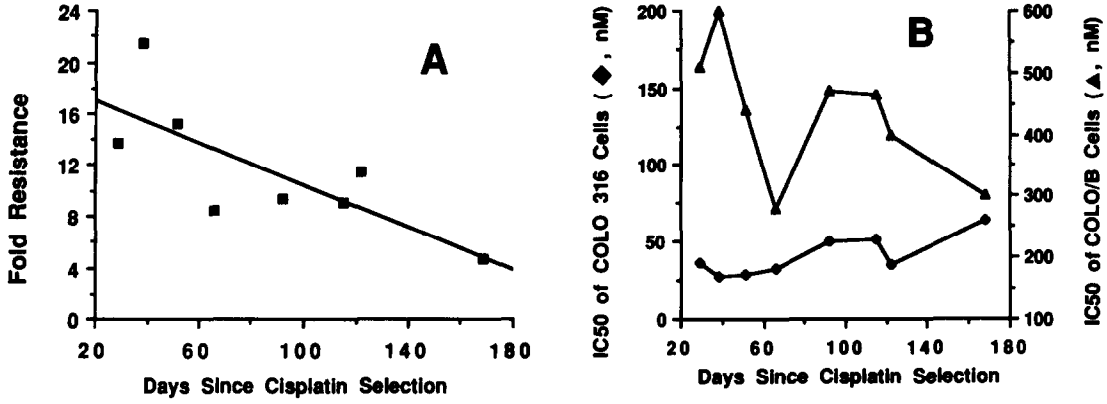

Fig. 2. Stability of DDP resistance in COLO 316 cells selected by intermittent high concentration exposure. After seven selections with 1.0 μM DDP, COLO/B cells were cultured in the absence of DDP and the resistance followed for 6 months. (A) Fold resistance, line calculated by linear regression. (B) Actual IC_{50} values for COLO 316 cells (◆); and for COLO/B cells (▲).

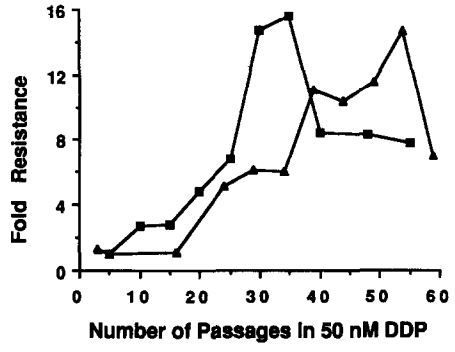

Fig. 3. Development of DDP resistance in COLO/DDP_{50} subline. COLO 316 cells were passaged twice per week in 50 nM DDP and the development of resistance determined by clonogenic assay. Lines represent two independent derivations.

Table 1. Flow cytometric analysis of COLO 316, COLO/B and COLO/DDP_{50} cells

Cell	Cell cycle compartment G_0/G_1	S	G_2/M	Forward scatter Mean channel No. ± SD
COLO 316	63.2%	33.8%	3.1%	36.9 ± 11.3
COLO/B	62.2	34.0	3.8	39.0 ± 12.6
COLO/DDP_{50}	57.7	36.3	6.0	37.9 ± 13.5

Cross-resistance

Cross-resistance to a variety of antitumor agents was determined for both sublines. Both COLO/B and COLO/DDP_{50} cells had high levels of cross-resistance to carboplatin (Table 2). Lower but significant cross-resistance to the analogs transDDP, DACH malonate and iproplatin was also found (Table 2). Both cell types were also cross-resistant to a variety of other DNA interactive drugs, including the alkylating agents mitomycin C, melphalan, and 4-hydroperoxycyclophosphamide, and the natural products etoposide, bleomycin and Adriamycin®. The major difference between these two cell types was seen against bleomycin. Collateral sensitivity was not seen to any of the agents tested.

DDP accumulation

Accumulation of platinum in cells exposed to 200 μM DDP was measured in subconfluent culture of the three cell types. Both COLO/D and COLO/DDP_{50} cells were 3.5-fold resistant at the time these studies were conducted. The accumulation after 1 h in the parent COLO 316 cells was 465 ± 76 pmol/mg protein ($n = 5$) (Table 3). After normalizing to minor differences in cell volumes, accumulation in the COLO/D and COLO/DDP_{50} cells were 52 and 101% of the parent cells respectively (Table 3).

GSH studies

COLO/B cells with 2–3-fold levels of resistance did not have elevated GSH [13]. When these cells were selected further for higher levels of resistance, however, small elevations in GSH were found (Table 4). At a level of 9-fold resistance the GSH was elevated 30% over that in parental cells. However, GSH levels in COLO/DDP_{50} that were 13-fold resistant were elevated 2.3-fold. The resistance of 3–6-fold resistant COLO/D and COLO/DDP_{50} cells could be partially reversed by depletion of GSH during DDP exposure (Table 4). However, GSH depletion did not return the sensitivity of resistant cells to that of the untreated parent cells. In addition, the effect was not specific for the resistant cells; GSH depletion produced similar DMFs in all cell types. Our previous reports on GSH depletion in these cells used a depletion protocol that removed the BSO at the time of DDP exposure [13, 15]. The prolonged GSH depletion protocol used here gave a DMF of 1.8 ± 0.1 ($n = 3$) for COLO 316 cells that was statistically different ($P < 0.01$) from our previously reported DMF of 1.2 ± 0.2 ($n = 6$) [15]. The DMF of 1.7 ± 0.4 ($n = 3$) obtained with prolonged GSH depletion

Table 2. Cross-resistance of COLO/B and COLO/DDP$_{50}$ cells to other antitumor agents. Resistance was determined by clonogenic assay on plastic and is reported as the ratio of the IC$_{50}$*s*

Drug	IC$_{50}$ in COLO 316*	Fold-resistance COLO/B	Fold-resistance COLO/DDP$_{50}$
DDP	0.05	11.4	7.9
transDDP	6.0	4.2	>4.2†
carboplatin	0.18	9.7	10.8
DACH malonate	0.27	3.0	3.5
iproplatin	0.66	2.5	4.5
melphalan	0.19	4.5	3.5
4-hydroperoxy-cyclophosphamide	0.28	2.4	2.1
mitomycin C	4.0 nM	>4.0†	3.4
etoposide	0.19	1.8	2.6
bleomycin	28 μU/ml	6.7	2.7
Adriamycin®	0.07 nM	2.1	2.3

*Reported as μM unless indicated otherwise.
†Indicates dose response line was insufficient to calculate an IC$_{50}$.

for COLO/D cells, however, was not statistically different ($P > 0.05$) from our previously reported value of 1.2 ± 0.2 ($n = 3$) for these cells [15].

DISCUSSION

We have developed DDP-resistant sublines of COLO 316 human ovarian carcinoma cells *in vitro* by two selection procedures. We chose selection procedures that may closely parallel conditions that select for DDP resistance *in vivo*. During i.p. chemotherapy, outer layers of ovarian carcinoma nodules are exposed to high concentrations of DDP. Inner regions of a tumor mass, however, may be exposed to much lower concentrations of DDP, due to poor vascularization and diffusion-limited delivery of DDP, following either i.v. or i.p. dosing. Since the biochemical mechanisms of DDP resistance that develop may be contingent upon the selection protocol, as has been found with methotrexate [17], we chose to select cells with both high and low concentrations of DDP. The number of exposures required to generate significant resistance, as well as the concentration of DDP used, were well within the scope of what is actually administered to patients. The development of resistance after intermittent selection with high concentrations of DDP was rapid, indicating, that if similar events transpire in the patient, then ovarian tumors have the potential for rapidly acquiring biochemical changes that protect them from DDP. The chronic selection procedure with low concentrations of DDP required a similar amount of time to develop significant resistance as the intermittent selection, although the actual number of fresh DDP exposures was much greater.

The apparent stability of the resistance indicated that the mechanisms could be attributable to heritable genetic alterations. However, no chromosomal changes could be detected by karyotypic analysis, as might be expected from the relatively low level of resistance examined. The DDP resistance could not be attributed to alterations in the growth characteristics of these cells since the doubling time, cloning efficiency and the percentage of cells in each cell cycle compartment were indistinguishable between resistant and parent cells.

The three curves in Fig. 1 showed variation during the development of resistance. With COLO/C cells the resistance decreased with further selection and in another case, COLO/D cells, high levels of resistance were not attained. The reasons for this variation are not known. However, one possibility may be the presence of mycoplasma. COLO/B and COLO/C cells were found to be mycoplasma contaminated at the eleventh and ninth selection and these cultures were terminated. It is possible that mycoplasma caused the loss of resistance in the COLO/B, COLO/C and COLO/DDP$_{50}$ cells. However, resistance also appeared to decline in COLO/D cells and these were free of mycoplasma. Nonetheless, we have observed no differences in the growth properties, the effects of GSH depletion on DDP sensitivity, or DDP accumulation in mycoplasma-contaminated versus mycoplasma-free cells.

COLO/D cells had markedly decreased drug accumulation compared to parent COLO 316 cells.

Table 3. Cisplatin accumulation and cell volumes of parent and cisplatin-resistant cells. Cells were exposed to 200 μM *cisplatin for* 1h *in RPMI* 1640 *medium. Volume was determined by the method of Kletzien* et al. [16]

Cell	Cell volume μl/mg protein (n)	DDP accumulation pmol/mg protein (n)	pmol/μl	%
COLO 316	8.5 ± 0.6 (3)	465 ± 76 (5)	54.7	100
COLO/D	9.1 ± 3.0 (3)	258 ± 120*(4)	28.4	52
COLO/DDP$_{50}$	8.1 ± 1.3 (3)	448 ± 77 (3)	55.3	101

*Significantly different from COLO 316 cells at $P < 0.05$, as determined by a t-test on grouped data.

Table 4. Glutathione levels in DDP-resistant cells and effect of glutathione depletion on DDP sensitivity

Cell	Fold-resistance	GSH level*	Fold-resistance	DMF† (*n*)
COLO 316	—	1.0	—	1.75 ± 0.08 (3)‡
COLO/B	7	1.3	—	—
COLO/D	—	—	3.5	1.70 ± 0.41 (3)§
COLO/DDP$_{50}$	13	2.3	3.5	1.57 ± 0.18 (4)

*Relative to parent COLO 316 cells with levels of 25–40 nmol/10^6 cells depending on confluency. Levels were determined on log-phase cells.

†Dose modification factor of GSH depletion, defined as IC_{50} control cells/IC_{50} GSH depleted cells.

‡Significantly different ($P < 0.01$) from previously reported value [15], as determined by a *t*-test on grouped data.

§Not significantly different ($P > 0.05$) from previously.reported value [15], as determined by a *t*-test on grouped data.

The fact that accumulation can be decreased by 50% suggests that the entry of DDP may involve more than simple passive diffusion, as was suggested to be the case for the dipyridine analog of DDP [18]. A growing body of evidence indicates that DDP accumulation may involve membrane transport mechanisms and that one target for DDP cytotoxicity may be the plasma membrane [19–22]. Numerous DDP-resistant sublines that have decreased DDP accumulation have now been reported [5, 6, 23–28]. The diminished accumulation of DDP is thus a common mechanism of DDP resistance. Altered DDP accumulation was clearly a contributing factor to the resistance of COLO/D cells, but, considering the degree of resistance attained, it is likely that there are other factors as well. Investigation of the mechanisms whereby DDP enters cells, and how these are modified in resistant cells, is an intriguing direction for further research.

In addition to their differences in DDP accumulation, COLO/D and COLO/DDP$_{50}$ cells varied in their GSH content. The level in the COLO/DDP$_{50}$ cells was much higher than either the COLO 316 or COLO/D cells. An explanation for this difference is that GSH may well be an important defense mechanism against continuous DDP exposure. Constant DDP treatment may keep GSH elevated as in COLO/DDP$_{50}$ cells, but when DDP is absent, as in COLO/D cells, GSH levels drift downward. These COLO/D cells must then remain resistant due to other factors, such as decreased DDP accumulation. It should be noted that COLO/D cells take 3–4 weeks to resume normal growth after intermittent high dose DDP exposure. At least a month had therefore elapsed since the last DDP selection before GSH levels, or any of the other studies described, were conducted. It is possible that GSH levels are more elevated immediately following selection in COLO/D cells. An alternative explanation for the difference in GSH levels may be that markedly elevated GSH develops in cells only after exposure to initially low concentrations of DDP. Acute exposure of cells to high concentrations of DDP may require cells to make more drastic alterations in their phenotype to protect themselves against this cytotoxic insult. Elevated GSH may not provide the protection needed to face such a serious selection pressure and thus is not expressed in COLO/D cells. Although the resistance of both cell types could be partially reversed by extended depletion of the GSH levels, this effect was not specific for the resistant cells. Parent COLO 316 cells also were significantly sensitized by this depletion protocol. These data indicate that, although elevated GSH may play some role in DDP resistance, there are also other mechanisms involved. The prolonged GSH depletion protocol used for these studies produced enhanced potentiation of DDP cytotoxicity, compared to our previous reports [13, 15]. This improvement of the DMF from 1.2 to 1.7 was small, however. We have also observed a similar enhancement of the DMF with the prolonged GSH depletion protocol in 2008 and 2008/DDP human ovarian carcinoma cells [29].

In summary, resistance to DDP can be rapidly developed in COLO 316 human ovarian carcinoma cells. Several biochemical mechanisms of resistance may arise and the relative importance of each appears to be influenced by the selection protocol. Resistance can be partially attributed to decreased accumulation and partially to elevated GSH. Other potential mechanisms of DDP-resistance remain to be investigated. The cell lines described in this report will be useful models for the conduct of these studies.

Acknowledgements—The technical assistance of Mr Mark A. Schiefer and Mr Hung H. Huynh and the assistance of the UCSD Flow Cytometry Facility are gratefully acknowledged.

REFERENCES

1. Loehrer PJ, Einhorn LH. Cisplatin. *Ann Intern Med* 1984, **100**, 704–713.
2. Goldie JH, Coldman AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res* 1984, **44**, 3643–3653.
3. Inoue EK, Mukaiyama T, Mitsui I, Ogawa M. *In vitro* evaluation of anticancer drugs in relation to development of drug resistance in the human tumor clonogenic assay. *Cancer Chemother Pharmacol* 1985, **15**, 208–215.
4. Ozols RF, Young RC. Chemotherapy of ovarian cancer. *Semin Oncol* 1984, **11**, 251–263.
5. Andrews PA, Velury S, Mann SC, Howell SB. *cis*-Diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res* 1988, **48**, 68–73.
6. Andrews PA, Murphy MP, Howell SB. Characterization of cisplatin-resistant COLO 316 human ovarian carcinoma cells. *Proc Am Ass Cancer Res* 1986, **26**, 289.
7. Hamilton TC, Winker MA, Louie KG *et al.* Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem Pharmacol* 1985, **34**, 2583–2586.
8. Behrens BC, Hamilton TC, Masuda H *et al.* Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987, **47**, 414–418.
9. Lu L, Lin Y, Han J, Scanlon KJ. Molecular and biochemical properties of cisplatin resistant A2780 ovarian carcinoma cells grown in folinic acid. *Proc Am Ass Cancer Res* 1987, **28**, 10.
10. Wolf CR, Hayward IP, Lawrie SS *et al.* Cellular heterogeneity and drug resistance in two ovarian adenocarcinoma cell lines derived from a single patient. *Int J Cancer* 1987, **39**, 695–702.
11. Kikuchi Y, Miyauchi M, Kizawa I, Oomori K, Kato K. Establishment of a cisplatin-resistant human ovarian cancer cell line. *J Nat Cancer Inst* 1986, **77**, 1181–1185.
12. Woods LK, Morgan RT, Quinn LA *et al.* Comparison of four new cell lines from patients with adenocarcinoma of the ovary. *Cancer Res* 1979, **39**, 4449–4459.
13. Andrews PA, Murphy MP, Howell SB. Differential potentiation of alkylating and platinating agent cytotoxicity in human ovarian carcinoma cells by glutathione depletion. *Cancer Res* 1985, **45**, 6250–6253.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 1976, **72**, 248–254.
15. Andrews PA, Murphy MP, Howell SB. Differential sensitization of human ovarian carcinoma and mouse L1210 cells to cisplatin and melphalan by glutathione depletion. *Mol Pharmacol* 1986, **30**, 643–650.
16. Kletzien RF, Pariza MW, Becker JE, Potter VR. A method using 3-*O*-methyl-D-glucose and phloretin for the determination of intracellular water space of cells in monolayer culture. *Anal Biochem* 1975, **68**, 537–544.
17. Rath H, Tlsty T, Schimke RT. Rapid emergence of methotrexate resistance in cultured mouse cells. *Cancer Res* 1984, **44**, 3303–3306.
18. Gale GR, Morris CR, Atkins LM, Smith AB. Binding of an antitumor platinum compound to cells as influenced by physical factors and pharmacologically active agents. *Cancer Res* 1973, **33**, 813–818.
19. Byfield JE, Calabro-Jones PM, Carrier-dependent and carrier-independent transport of anti-cancer alkylating agents. *Nature* 1981, **294**, 281–283.
20. Scanlon KJ, Safirstein RL, Thies H *et al.* Inhibition of amino acid transport by *cis*-diamminedichloroplatinum(II) derivatives in L1210 murine leukemia cells. *Cancer Res* 1983, **43**, 4211–4215.
21. Gross RB, Scanlon KJ. Amino acid membrane transport properties of L1210 cells resistant to cisplatin. *Chemioteripia* 1986, **5**, 37–43.
22. Dornish JM, Melvik JE, Pettersen EO. Reduced cellular uptake of *cis*-dichlorodiammineplatinum by benzaldehyde. *Anticancer Res* 1986, **6**, 583–588.
23. Hromas RA, North JA, Burns CP. Decreased cisplatin uptake by resistant L1210 leukemia cells. *Cancer Lett* 1987, **36**, 197–201.
24. Richon VM, Schulte N, Eastman A. Multiple mechanisms of resistance to *cis*-diamminedichloroplatinum(II) in murine leukemia L1210 cells. *Cancer Res* 1987, **47**, 2056–2061.
25. Waud WR. Differential uptake of *cis*-diamminedichloroplatinum(II) by sensitive and resistant murine L1210 leukemia cells. *Cancer Res* 1987, **47**, 6549–6555.
26. Kraker AJ, Moore CW. Accumulation of *cis*-diamminedichloroplatinum(II) and platinum analogs by platinum-resistant murine leukemia cells *in vitro*. *Cancer Res* 1988, **48**, 9–13.
27. Wallner KE, Degregorio MW, Li GC. Hyperthermic potentiation of *cis*-diamminedichloroplatinum(II) cytotoxicity in Chinese hamster ovary cells resistant to the drug. *Cancer Res* 1986, **46**, 6242–6245.
28. Teicher BA, Holden SA, Kelley SA *et al.* Characterization of a human squamous carcinoma cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1987, **47**, 388–393.
29. Andrews PA, Scheifer MA, Murphy MP, Howell SB. Enhanced potentiation of cisplatin cytotoxicity in human ovarian carcinoma cells by prolonged glutathione depletion. *Chem-Biol Interact* 1988, **65**, 51–58.