Cancer Chemother Pharmacol (1997) 40: 159–166

ORIGINAL ARTICLE

D. E. Wilkins · C. E. Ng · G.P. Raaphorst

Cell cycle perturbations in cisplatin-sensitive and resistant human ovarian carcinoma cells following treatment with cisplatin and low dose rate irradiation

Received: 22 December 1995 / Accepted: 28 September 1996

Abstract *Purpose*: To investigate cell cycle pertubations in plateau-phase human ovarian carcinoma cells following treatment with cisplatin, low dose-rate irradiation (LDRI), or combined cisplatin and LDRI, in order to understand cell cycle mechanisms by which these two treatment modalities interact. *Methods*: Human ovarian carcinoma cells sensitive (A2780) and resistant (2780^{CP}) to cisplatin were grown to plateau phase and given protracted cisplatin treatments (A2780 0.7 and 2 μg/ml; 2780^{CP} 5 and 15 μg/ml) and/or LDRI (0.41 cGy/min). Cell cycle distribution following treatment was determined by two-parameter flow cytometry, based on bromodeoxyuridine (BrdU) uptake and DNA content using propidium iodide staining. *Results*: The cisplatin-sensitive A2780 cells exposed to cisplatin alone for up to 28 h showed depletion of the G1 fraction and accumulation in S-phase, although the percentage of S-phase cells actively incorporating BrdU dropped to almost zero. The cisplatin-resistant 2780^{CP} cells exposed to cisplatin alone showed a G1 arrest when exposed to 15 μg/ml, but not when exposed to 5 μg/ml. LDRI alone caused little cell cycle redistribution different from controls in either cell line. When LDRI was combined with cisplatin, no significant cell cycle redistribution was observed, apart from a decline in the actively incorporating S-phase fraction. *Conclusions*: Cisplatin caused A2780 cells to accumulate in nonincorporating S-phase, with no evidence of G1 arrest. Cisplatin-resistant 2780^{CP} cells showed a G1 block when exposed to a high enough cisplatin concentration. This could indicate a mechanism of cisplatin resistance in these cells. LDRI alone or in combination with cisplatin did not result in significant cell cycle redistribution.

D.E. Wilkins (✉) · C.E. Ng · G.P. Raaphorst
Ottawa Regional Cancer Centre, 190 Melrose Avenue,
Ottawa, Canada K1Y 4K7
Tel. 613-247-6826;
Fax. 613-725-6395; E-mail dwilkins@OCTRF.on.ca

Key words Cisplatin · Low dose-rate irradiation · Cell cycle

Introduction

Cisplatin has been shown to be a radiation-sensitizing agent in human cancer cell lines, possibly acting through inhibition of repair of potentially lethal and sublethal damage [5, 6, 21]. Under conditions of low dose-rate irradiation (LDRI), such as in brachytherapy or radioimmunotherapy, repair of sublethal and potentially lethal radiation damage occurs [7, 18] and repair-inhibiting radiosensitizers such as cisplatin could play a major role in the treatment of repair-proficient tumors. While the cytotoxic effects of cisplatin are generally considered to be noncell-cycle specific [16], cisplatin can cause perturbations in cell cycle distribution [10]. This can be important when cisplatin is combined with radiation, particularly at dose rates below 2 cGy/min, where cell cycle progression can have a significant effect on radiosensitivity [18] .

The effects of cisplatin on cell cycle distribution have been investigated in a number of human cancer cell lines. Like many DNA-damaging agents, cisplatin can induce G2 arrest in some cell lines, which can then be followed by apoptosis [4]. Cisplatin has been shown to increase the fraction of cells in S-phase in two prostate cancer cell lines [12], and has been reported to cause an S-phase block in two human ovarian carcinoma cell lines [10]. This could be significant in combined cisplatin and radiation treatments because S-phase is generally considered to be a radioresistant phase of the cell cycle, although sensitivity in early S-phase has been reported [8].

Single-parameter flow cytometry using propidium iodide to determine cell cycle distribution based on DNA content cannot identify S-phase cells which are not actively synthesizing DNA. Dual-parameter flow cytometry measuring both DNA content and incorporation of BrdU must be used to identify these cells [22].

In studies involving cisplatin and radiation, cisplatin has been shown to induce accumulations of human ovarian carcinoma cells in S-phase which are not actively synthesizing DNA [11]. Radiation causes similar nonincorporating accumulations in S-phase in human melanoma cells [23], indicating the importance of dual-parameter flow cytometry in detecting cell cycle perturbations caused by cisplatin and radiation.

Many studies have examined in vitro cell cycle perturbations of cisplatin and LDRI using cells in exponential growth phase. However, most solid tumors contain a large fraction of cells which are not actively cycling, and there are few in vitro data concerning cell cycle redistribution of plateau-phase cells following LDRI or cisplatin treatment. Generally, plateau-phase cells are used experimentally to minimize cell cycle effects, but most cell lines exhibit residual progression even in the plateau phase. In this in vitro study, plateau-phase human ovarian carcinoma cells sensitive (A2780) and resistant (2780^{CP}) to cisplatin were used to investigate cell cycle perturbations following treatment with LDRI (0.41 cGy/min) and cisplatin, in order to improve our understanding of the interaction of these two treatment modalities. The measurements were restricted to those cells which remained attached to the growing surface throughout the treatment, since these cells represent those most likely to survive in vivo treatments and go on to repopulate a tumor, possibly resulting in treatment failure.

Materials and methods

Human ovarian carcinoma cells A2780, and their cisplatin-resistant variant 2780^{CP}, were grown in a 1:1 mixture of DMEM/F12 with 7.5% fetal bovine serum (FBS) and 7.5% newborn calf serum NCS. Cells were seeded at a density of 8×10^3 cells/cm^2 in either T-25 flasks (for cisplatin treatments alone) or in small glass vials with 0.5 ml medium (for LDRI treatments). Cells were refed on day 5, and experiments started on day 8, when the cultures were in plateau phase (greater than 75% of cells in G0/G1 phase).

LDRI was performed in a ^{226}Ra cellular irradiator [13] which could hold ten small glass vials of cells. The irradiator was in a temperature-controlled incubator maintained at 37 °C. The dose rate was 0.41 cGy/min (12 Gy in 48 h). Cisplatin Injection (1 mg/ml cisplatin in 0.9% NaCl) was diluted 1:10 in citrate saline and added directly to the medium covering the cells. Equal volumes of saline without cisplatin were added to control (untreated) cells. At the end of the incubation at 37 °C with cisplatin, cells were rinsed twice with citrate saline to remove the cisplatin prior to trypsinization.

Cell cycle analysis was performed by adding bromodeoxyuridine (10 μM BrdU) directly to the medium for the last 30 min of treatment. Cells were then rinsed twice, trypsinized, resuspended in medium, centrifuged at 4 °C, washed with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), resuspended in citrate saline on ice and fixed in 70% ethanol on ice for 30 min. The cells were incubated at room temperature for 30 min in 2 N HCl/Triton X-100, which was neutralized with 0.1 M $Na_2B_4O_7$. Tween 20 (0.5% v/v) in 1% BSA/PBS containing RNAase (50 μg/ml) and anti-BrdU-FITC conjugate was added for 30 min, and propidium iodide (5 μg/ml) was added just prior to running flow cytometry [1]. Two-parameter flow cytometry was performed on a Coulter Epics XL flow cytometer, with approximately 5×10^4 cells measured from each sample. Data was analyzed using Multicycle software (Pheonix Flow Systems). Each experiment was repeated two or three times; plotted points are means ± SEM.

Data analysis was performed by first fitting each data set as a one-parameter histogram (cell number versus propidium iodide fluorescence). From this, the total S-phase fraction was determined, i.e. the fraction of cells with DNA content intermediate between diploid and tetraploid, as well as the fraction of cells in G0/G1 and G2/M. A two-parameter histogram was then plotted (FITC fluorescence versus propidium iodide fluorescence), and a region was drawn around those cells that had incorporated BrdU, as determined by their FITC fluorescence. Discrimination between those cells that did and those that did not incorporate BrdU was determined by comparison with controls which had not been exposed to BrdU but which had been exposed to anti-BrdU-FITC. The incorporating cells were then subtracted from the population, and the remaining cells refitted according to a one-parameter fit based on propidium iodide fluorescence. The S-phase fraction of this second fit, after normalization to the total cell number before subtraction, was the nonincorporating S-phase fraction. The active S-phase fraction was the total S-phase fraction from the first fit minus the nonincorporating S-phase fraction determined from the second fit.

Results

Figure 1 shows examples of two-parameter flow cytometry histograms for control (untreated) A2780 cells and cells treated with 0.7 μg/ml cisplatin for 24 h. The propidium iodide fluorescence (associated with DNA content) is along the horizontal axis, with FITC fluorescence (associated with BrdU incorporation) along the vertical axis. The control plot shows that of the cells with S-phase DNA content, most had incorporated BrdU and showed FITC fluorescence. In contrast, most of the cisplatin-treated cells with S-phase DNA content by propidium iodide fluorescence did not show FITC fluorescence, indicating the presence of a nonincorporating S-phase fraction.

Results of flow cytometric cell cycle analysis of A2780 cells following continuous exposure to 0.7 μg/ml cisplatin are shown in Fig. 2. The clonogenic survival after 12 h of exposure to this concentration of cisplatin was 5% for A2780 cells. The control cells incubated at 37 °C with no cisplatin exposure showed increasing accumulation in G0/G1 as the cells moved deeper into the plateau phase. (Fig. 2a) Cells exposed to cisplatin appeared to move out of G0/G1 and into S-phase, where the total S-phase fraction increased from 10% to 35% (Fig. 2b). The cells accumulated in S-phase as the fraction moving from S-phase into G2/M was reduced. The accumulation in S-phase was predominantly nonincorporating cells. The active S-phase fraction decreased with time of cisplatin exposure from 18% to about 8%, suggesting that the cells were unsuccessful at DNA synthesis, and were increasingly unable to incorporate BrdU.

In A2780 cells exposed to a higher cisplatin concentration (2 μg/ml, giving a clonogenic survival of approximately 0.1% after 12 h exposure), a similar decrease in G0/G1 was observed with a concomitant increase in total S-phase fraction (Fig. 3). The actively incorporating S-phase fraction dropped quickly to near zero, and by the end of the treatment almost all S-phase cells were non-incorporating. At this higher cisplatin

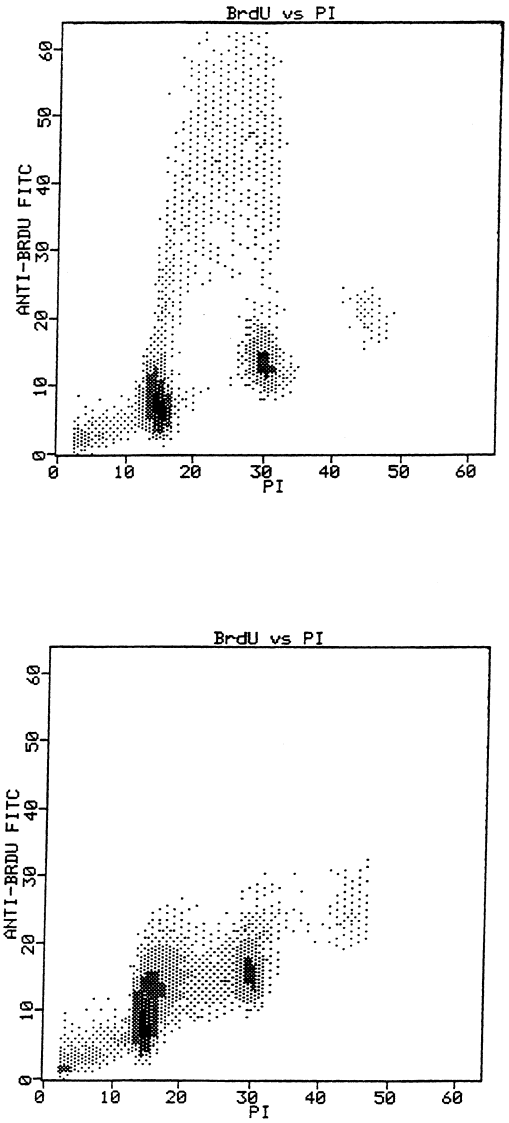


Fig. 1 Sample two-parameter flow cytometry histograms for the parental A2780 human ovarian carcinoma cells. (*Upper* control untreated cells, *lower* cells treated for 24 h with 0.7 μg/ml cisplatin)

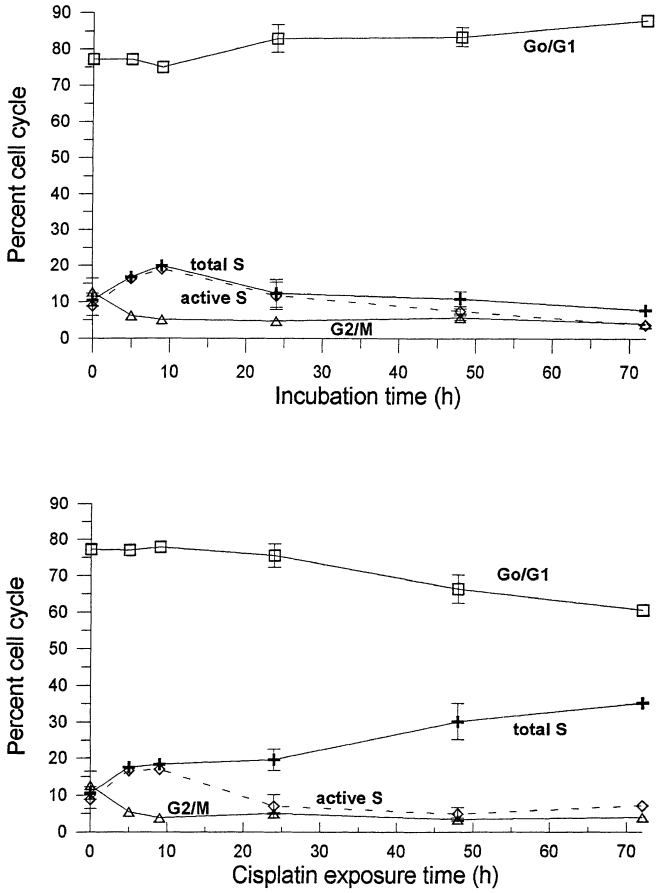


Fig. 2 Cell cycle redistribution of plateau-phase A2780 cells. (*Upper* control untreated cells incubated at 37 °C, *lower* cells continuously exposed to 0.7 μg/ml cisplatin)

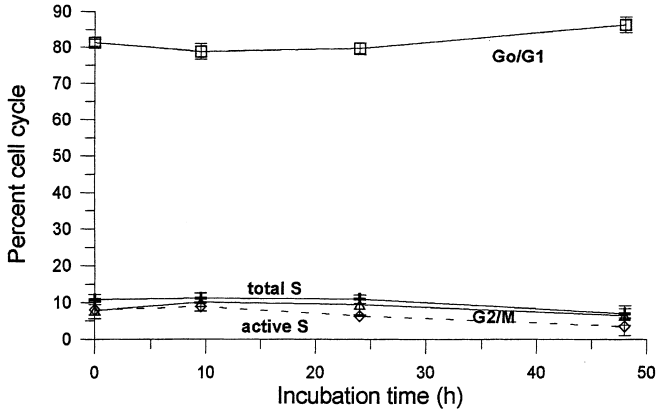
concentration, the accumulation in S-phase was less pronounced than at 0.7 μg/ml, suggesting that progression through the G1/S boundary was somewhat inhibited by 2 μg/ml compared with 0.7 μg/ml cisplatin.

The cisplatin-resistant variant 2780^{CP} was exposed to 5 μg/ml cisplatin (a level of exposure approximately equitoxic to 0.7 μg/ml in A2780 cells, with clonogenic survival of approximately 5% after 12 h of exposure), and the results are shown in Fig. 4. As with 0.7 μg/ml in the parental A2780 cells, there was movement across the G1/S boundary and accumulation of cells in S-phase. The total S-phase fraction increased from 10% to 20%, while the G0/G1 fraction decreased from a peak of 85% to 65% at 72 h. The actively incorporating S-phase fraction quickly dropped to near zero, as the increase in S-phase fraction was a consequence of an increase in nonincorporating S-phase cells. There was no decrease in the G2/M fraction, suggesting a possible G2 block which prevented depletion of the G2/M pool.

The behaviour of 2780^{CP} cells exposed to the higher cisplatin concentration of 15 μg/ml (approximately equitoxic to 2 μg/ml in A2780 cells, with a clonogenic survival of approximately 0.1% after 12 h exposure) was quite different (Fig. 5). The G0/G1 fraction increased from 78% to 92%, and the total S-phase fraction decreased from a peak of 15% to 5%, indicating cisplatin-induced G1 arrest. The actively incorporating S-phase fraction quickly dropped to near zero.

Parental A2780 cells were subjected to LDRI (0.41 cGy/min) (Fig. 6). At this dose rate, a clonogenic survival level of 1% was reached at a dose of 9 Gy for A2780 cells, and at 11 Gy for 2780^{CP} cells. There was no clear evidence of cell cycle redistribution in these cells during LDRI. A slight decline in actively incorporating S-phase fraction indicated that these cells were increasingly unable to successfully synthesize DNA under these conditions of LDRI. Similarly, 2780^{CP} cells subjected to LDRI seemed to show no cell cycle redistribution significantly different from control cells incubated at 37 °C in the absence of LDRI (Fig. 7).

Parental A2780 cells treated with concurrent LDRI and 0.7 μg/ml cisplatin (Fig. 8) also showed little



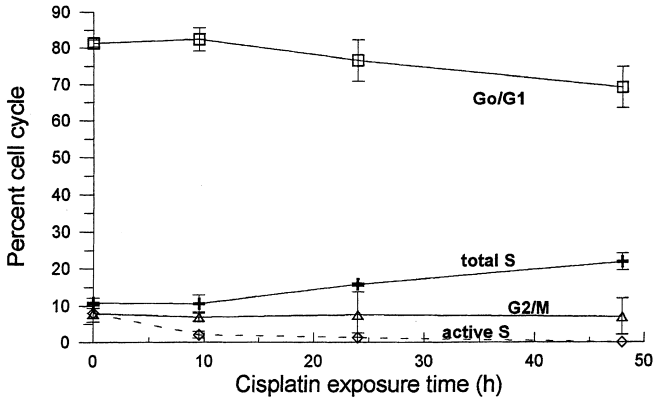
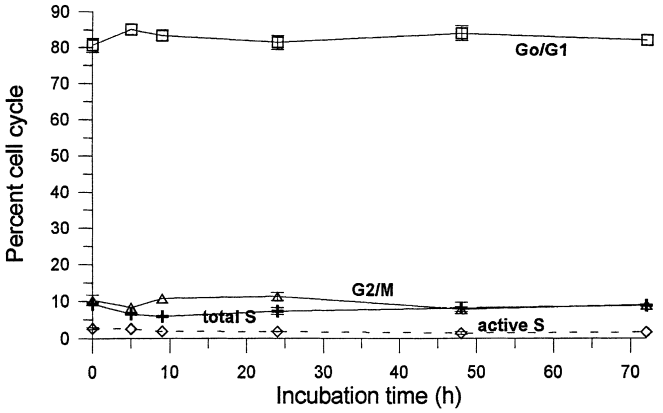


Fig. 3 Cell cycle redistribution of plateau-phase A2780 cells. (*Upper* control untreated cells, *lower* cells exposed to 2 μg/ml cisplatin)



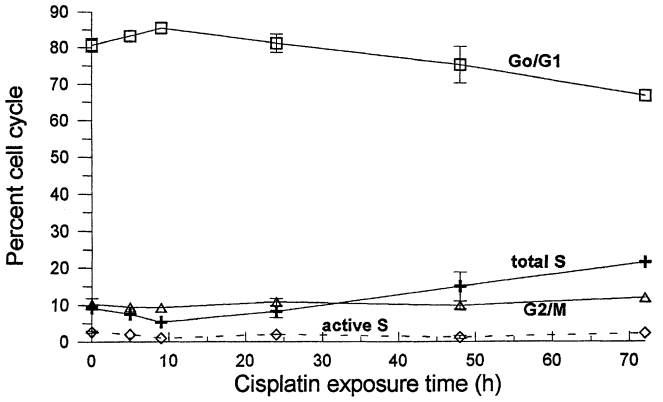


Fig. 4 Cell cycle redistribution of cisplatin-resistant 2780^{CP} cells in plateau phase (**a** control untreated cells, **b** cells exposed to 5 μg/ml cisplatin)

evidence of cell cycle redistribution different from controls, except for a decrease in the active S-Phase fraction. Similarly, the cisplatin-resistant 2780^{CP} treated with concurrent LDRI and 5 μg/ml cisplatin (Fig. 9) showed little redistribution different from controls beyond a decrease in the active S-phase fraction. It should be emphasized that the samples were rinsed twice with citrate saline at the end of the treatment period to remove the cisplatin and BrdU, so the cells assayed for cell cycle distribution were attached cells only. The number of cells harvested in this way from each vial treated with concurrent LDRI and cisplatin was only about half the number of cells harvested from vials receiving cisplatin only or LDRI only, despite the same number of cells at the beginning of the treatment. This indicates a higher amount of cell killing by concurrent LDRI and cisplatin, resulting in a large number of floating cells.

Discussion

One of the factors mediating the response of cells to DNA-damaging agents is their p53 status [9]. Both A2780 parental and cisplatin-resistant 2780^{CP} cells have been found to have wildtype p53, but the cisplatin-resistant clones frequently show increased levels of expression of the p53 protein [2]. It has been suggested [9] that the presence of G1 arrest induced by DNA-damaging agents may be an important measure of p53 function. By this measure, our cisplatin data (Figs. 4 and 5) agree with the measurements of Brown et al. showing increased levels of wildtype p53 in cisplatin-resistant clones of A2780 cells [2]. In our experiments, the parental A2780 (cisplatin-sensitive) cells showed no evidence of a G1 arrest after exposure to cisplatin. It is possible that the high level of p53 in the 2780^{CP} cells helped to confer resistance on these cells by allowing time during the G1 arrest to repair damage inflicted by the cisplatin. It has been shown that transfection of the mutant p53 gene construct into cisplatin-resistant cells makes them more sensitive to cisplatin, whereas transfecting mutant p53 into parental A2780 does not change cisplatin resistance, suggesting that p53 can be directly involved in resistance [2]. It should be noted that the 2780^{CP} cells also show increased resistance to high dose-rate irradiation [15] compared with the parental A2780 cells.

In the cisplatin-resistant 2780^{CP} cells, exposure to 5 μg/ml cisplatin did not cause G1 arrest, whereas exposure to 15 μg/ml did. This indicates a dose dependence for cisplatin-induced G1 arrest, and perhaps a threshold

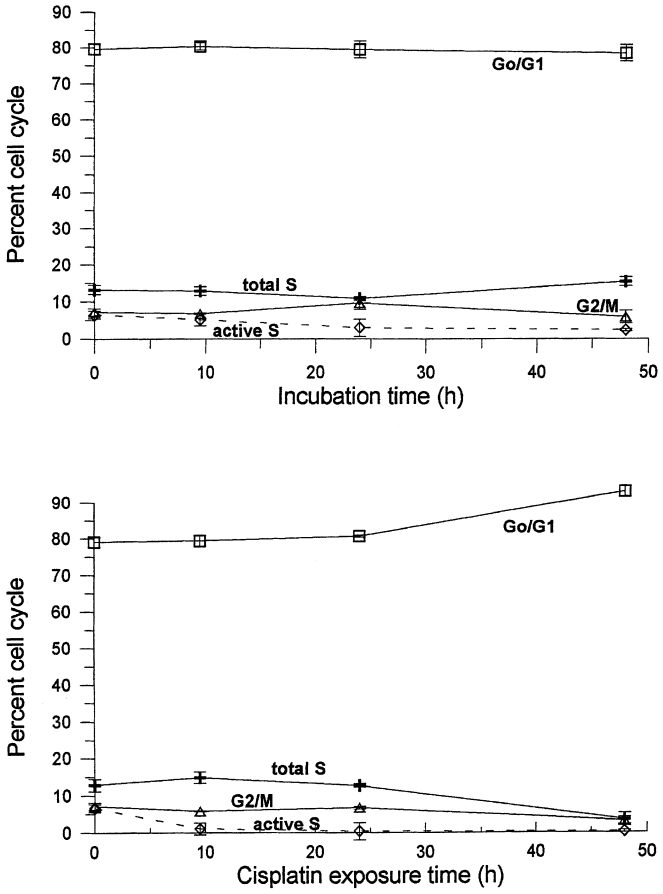


Fig. 5 Cell cycle redistribution of plateau-phase cisplatin-resistant 2780^{CP} cells (**a** control untreated cells, **b** cells exposed to 15 μg/ml cisplatin)

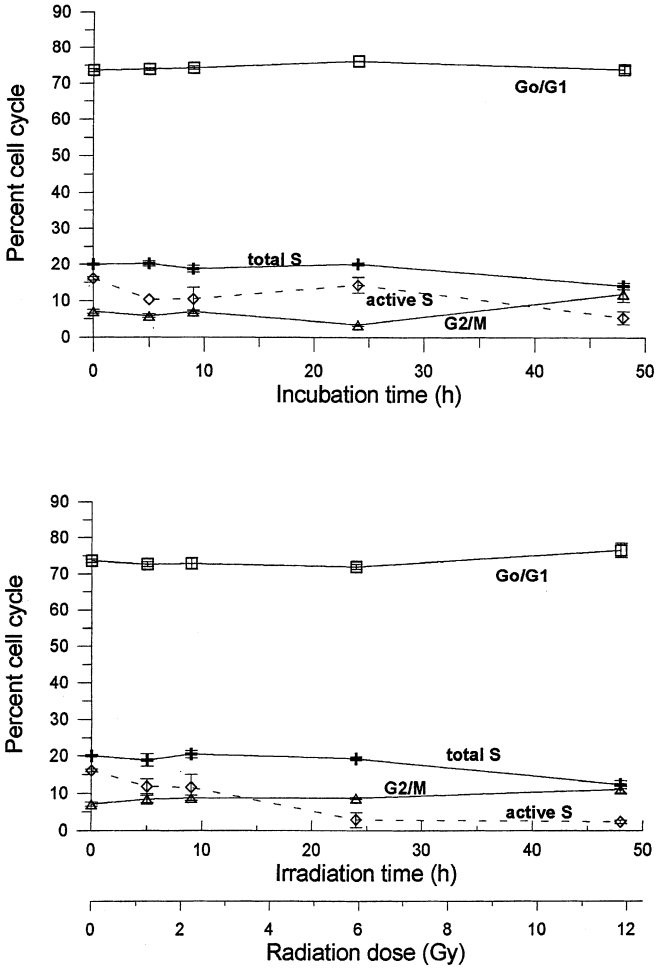


Fig. 6 Cell cycle redistribution of parental A2780 cells. (*Upper* control untreated cells, *lower* cells treated with continuous LDRI 0.41 cGy/min)

of cisplatin-induced DNA damage which must be sustained before the G1 arrest is triggered. It should be noted that a cisplatin concentration of 15 μg/ml is higher than is routinely achieved clinically; 5 μg/ml is more representative of plasma cisplatin concentrations during treatment [17, 19].

In the absence of G1 arrest, A2780 cells exposed to 0.7 and 2 μg/ml cisplatin, and 2780^{CP} cells exposed to 5 μg/ml cisplatin showed depletion of the G1 fraction and accumulation of cells in S-phase. Most of these S-phase cells were not incorporating BrdU at the end of the treatment, indicating they were not actively synthesizing DNA at that time. Earlier time-points showed the presence of an incorporating (active) S-phase fraction. It is likely that the progression of G1 cells into this active S-phase fraction, and subsequent halting of DNA synthesis by the cytotoxic effects of the treatment, caused accumulation of cells in the nonincorporating S-phase fraction. The pulse-labelling by BrdU at the end of the treatment labelled only the small active S-phase fraction, not the cells with S-phase DNA content which were no longer actively synthesizing DNA.

Accumulation in S-phase has also been reported by Qiao et al. in two prostate carcinoma cell lines [12], and by Nguyen et al. in two ovarian carcinoma cell lines [10] following cisplatin exposure. Our flow cytometry histograms showed that the S-phase accumulation was predominantly in early S-phase; the histograms produced by Qiao et al. also show accumulation in early S-phase [12]. Our results do not show whether the accumulation in S-phase was temporary or reversible, or whether it represented a permanent condition in those cells destined to succumb to the cytotoxic effects of cisplatin.

A common feature of all the treatments, cisplatin and radiation, was the rapid accumulation of a nonincorporating S-phase fraction. This fraction was also seen in the control (untreated) populations, although to a much lower extent. As the control cells progressed deeper into the plateau phase (characterized by increased cell density, nutrient depletion and lowered pH), there was generally an increasing difference between the total S-phase fraction and the actively incorporating S-phase fraction. A nonincorporating S-phase fraction has also been reported by Tinnemans et al. in untreated nutrient-deprived lung cancer cells, associated with a reduction in vitality of the culture as measured by trypan blue exclusion [20]. The ultimate fate of cells in the nonincorporating S-phase

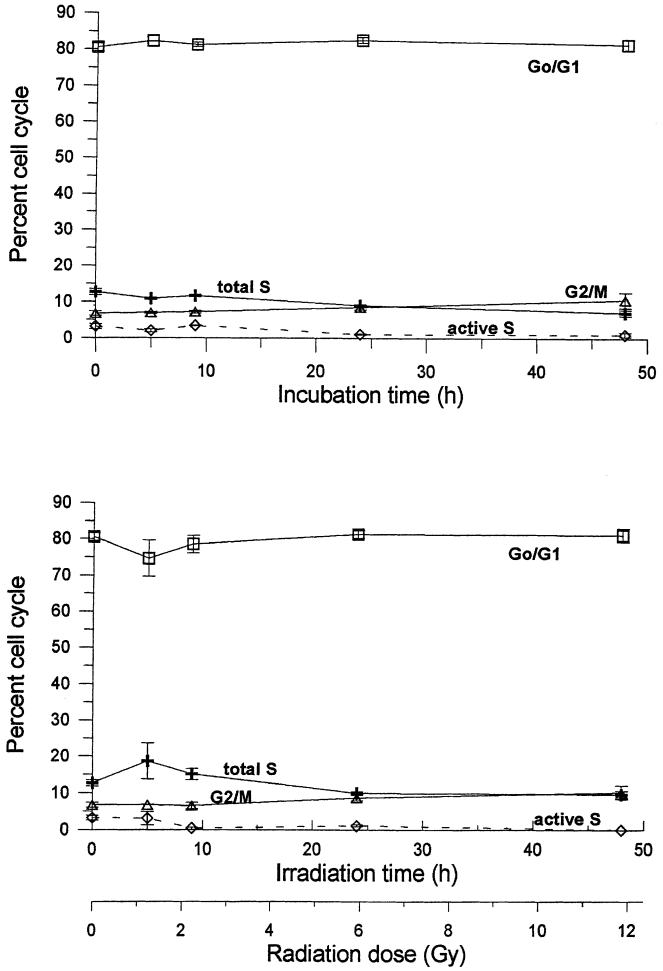


Fig. 7 Cell cycle redistribution of cisplatin resistant 2780^{CP} cells. (*Upper* control untreated cells, *lower* cells treated with continuous LDRI 0.41 cGy/min)

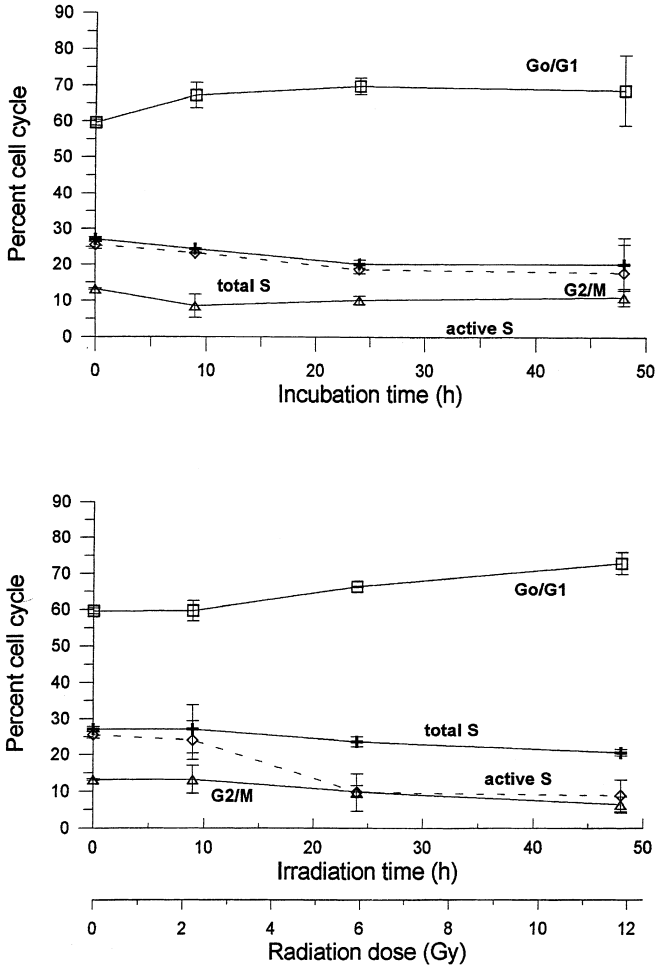


Fig. 8 Cell cycle redistribution of parental A2780 cells. (*Upper* control untreated cells, *lower* cells treated with combined cisplatin 0.7 μg/ml and continuous LDRI 0.41 cGy/min)

fraction can only be determined by cell sorting and subsequent clonogenic survival assay of these cells; this was not done either in the study by Tinnemans et al. or in this study. Pulse-chase experiments have revealed that cells in the nonincorporating S-phase fraction (as induced by nutrient depletion) can re-enter the cell cycle, depending on the duration of the S-phase arrest. However, it is uncertain whether the nonincorporating S-phase fractions induced by the cytotoxic treatments used in the present study are reversible.

LDRI (0.41 cGy/min) caused no cell cycle redistribution in either cell line. No evidence of cell cycle blocks in G1 or G2/M evident from Figs. 6 or 7. At this dose rate, it is likely that most radiation-induced single-strand breaks were fully repaired, resulting in survival curves with little or no shoulder [14]. Since induction of increased p53 levels following ionizing radiation may be triggered by DNA single-strand breaks, and radiation-induced G1 arrest is mediated by p53 [3], the likely full repair of single-strand breaks under these conditions of LDRI would not be expected to lead to a G1 arrest.

When LDRI was combined with concomitant cisplatin treatment, no significant cell cycle redistribution effects were observed in either cell line. It would appear that the accumulation in S-phase observed in A2780 cells with exposure to 0.7 μg/ml cisplatin (Fig. 2) was abrogated by the addition of LDRI. The most likely explanation is that those cells that accumulated in the nonincorporating S-phase fraction as a result of exposure to cisplatin became nonattached cells when concomitant LDRI was present. Only attached cells were harvested for cell cycle analysis. The number of cells harvested was not significantly lower than controls when only cisplatin or LDRI treatments were applied individually, but when cisplatin and LDRI were combined, the number of harvested cells at the end of the treatment dropped to approximately half that of controls for both cell lines. It is likely that some of the elevated nonincorporating S-phase fraction became detached during LDRI, but further experiments analyzing the distribution of the detached cell pool are necessary to confirm this.

When exposed to cisplatin, the parental A2780 cells showed a tendency to accumulate in the nonincorpo-

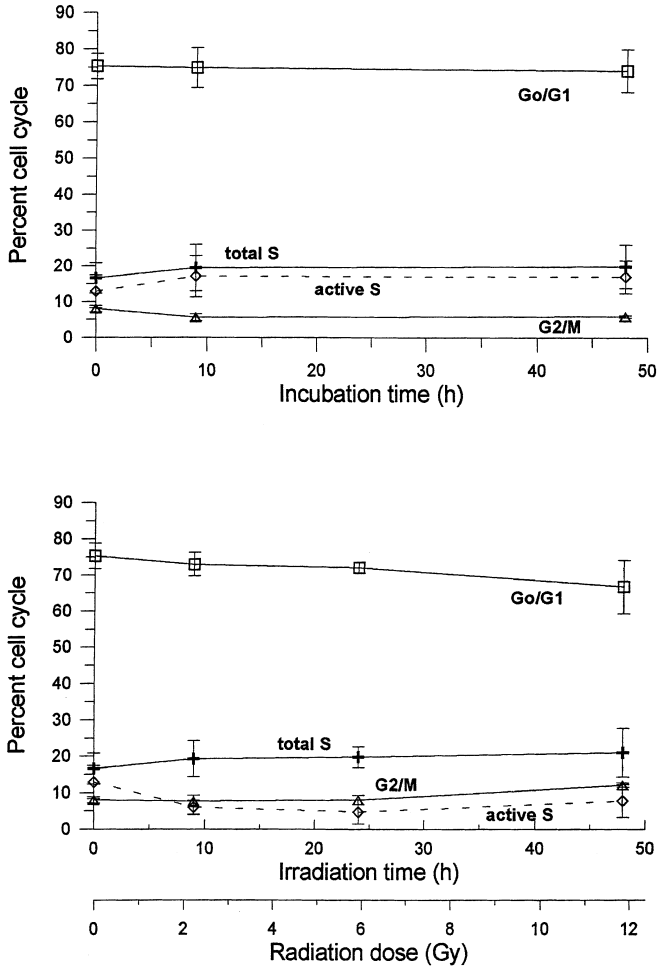


Fig. 9 Cell cycle redistribution of cisplatin-resistant 2780^{CP} cells. (*Upper* control untreated cells, *lower* cells treated with combined cisplatin 5 μg/ml and continuous LDRI 0.41 cGy/min)

rating S-phase, with little evidence of a G1 block. In contrast, the cisplatin-resistant 2780^{CP} cells did show evidence of a G1 block at the higher cisplatin concentration of 15 μg/ml. This is consistent with reports of higher levels of wildtype p53 expression in 2780^{CP} cells compared with the parental A2780, with p53 implicated in G1 block. LDRI at 0.41 cGy/min did not result in any measurable cell cycle redistribution, with this dose rate possibly too low to cause radiation-induced G2 block in these plateau-phase cells. When continuous cisplatin exposure was combined with LDRI, neither cell line showed evidence of cell cycle redistribution different from controls. However, the number of attached cells was reduced to half that of controls, indicating that those cells previously blocked in nonincorporating S-phase (A2780) or G1 (2780^{CP}) by cisplatin treatment alone were likely rendered nonviable and detached from the growing surface when LDRI was added.

Cell cycle redistributions in plateau-phase cells resulting from these in vitro treatments were modest compared with what might be expected in exponentially growing cell cultures. Nevertheless, redistributions were clearly measurable and potentially of clinical significance, particularly for the cisplatin treatments alone. The lack of significant cell cycle perturbations when cisplatin and LDRI were combined suggests that cisplatin-induced accumulation in S-phase does not result in increased radiation resistance of ovarian carcinoma cells. These experiments clearly show the importance of two-parameter cell cycle analysis, since the cisplatin-induced accumulation in S-phase observed in A2780 cells was the result of an increase in nonincorporating cells with S-phase DNA content.

Acknowledgements This work was supported by a grant from the National Cancer Institute of Canada.

References

1. Becton Dickinson Immunocytometry Systems (1993) Monoclonal antibodies detecting cell proliferation: anti-bromodeoxyuridine. Sourcebook section 3.80.1
2. Brown R, Clugston C, Burns P, Edlin A, Vasey P, Vojtesek B, Kaye SB (1993) Increased accumulation of p53 protein in cisplatin-resistant ovarian cell lines. Int J Cancer 55: 678
3. Canman CE, Wolff AC, Chen C-Y, Fornace AJ, Kastan M (1994) The p53-dependent G1 cell cycle checkpoint pathway and ataxia-telangiectasia. Cancer Res 54: 5054
4. Chu G (1994) Cellular responses to cisplatin: the roles of DNA-binding proteins and DNA repair. J Biol Chem 269: 787
5. Dewit L (1987) Combined treatment of radiation and cis-diaminedichloroplatinum(II): a review of experimental and clinical data. Int J Radiat Oncol Biol Phys 13: 403
6. Douple EB (1990) Interactions between platinum coordination complexes and radiation. In: Hill BT, Bellamy AS (eds) Antitumour drug-radiation interactions, CRC Press, Boca Raton, p 172–190
7. Hall EJ, Brenner EJ (1991) The dose-rate effect revisited: radiobiological considerations of importance in radiotherapy. Int J Radiat Oncol Biol Phys 21: 1403
8. McGinn CJ, Miller EM, Lindstrom MJ, Kunugi KA, Johnston PG, Kinsella TJ (1994) The role of cell cycle redistribution in radiosensitization: implications regarding the mechanism of fluorodeoxyuridine radiosensitization. Int J Radiat Oncol Biol Phys 30: 851
9. McIlwrath AJ, Vasey PA, Ross GM, Brown R (1994) Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type p53 for radiosensitivity. Cancer Res 54: 3718
10. Nguyen HN, Sevin BU, Averette HE, Perras J, Ramos R, Donato D, Ochiai K, Penalver M (1993) Cell cycle perturbations of platinum derivatives on two ovarian cancer cell lines. Cancer Invest 11: 264
11. Perras JP, Ramos R, Sevin BU (1993) Demonstration of an S phase population of cells without DNA synthesis generated by cisplatin and pentoxifylline. Cytometry 14: 441
12. Qiao L, Pizzolo G, Melamed MR (1994) Effects of selected chemotherapeutic agents on PCNA expression in prostate carcinoma cell lines. Urol Res 22: 171
13. Raaphorst GP, Bussey A, Heller DP, Ng CE (1994) Comparison of thermoradiosensitization in two human melanoma cell lines and one fibroblast cell line by concurrent mild hyperthermia and low-dose-rate irradiation. Radiat Res 137: 338
14. Raaphorst GP, Heller DP, Bussey A, Ng CE (1994) Thermal radiosensitization by 41 °C hyperthermia during low dose-rate irradiation in human normal and tumour cell lines. Int J Hyperthermia 10: 263
15. Raaphorst GP, Wang G, Wilkins D, Davis L, Bussey A, Stewart D, Ng CE (1994) Evaluation of cisplatin response in

three radiation resistant and three radiation sensitive cell lines. Anticancer Res 14: 1939
16. Schweitzer VG (1993) Cisplatin-induced ototoxicity: the effect of pigmentation and inhibitory agents. Laryngoscope 103: 1
17. Sileni VC, Fosser V, Maggian P, Padula E, Beltrame M, Nicolini M, Arslan P (1992) Pharmacokinetics and tumor concentration of intraarterial and intravenous cisplatin in patients with head and neck squamous cancer. Cancer Chemother Pharmacol 30: 221
18. Steel GG, Down JD, Peacock JH, Stephens TC (1986) Dose-rate effects and the repair of radiation damage. Radiother Oncol 5: 321
19. Stewart DJ, Mikhael NZ, Nair RC, Kacew S, Montpetit V, Nanji A, Maroun JA, Howard K (1988) Platinum concentrations in human autopsy tumor samples. Am J Clin Oncol 11: 152
20. Tinnemans MMJF, Lenders M-H, ten Velde GPM, Blijham GH, Ramaekers FCS, Schutte B (1995) S-phase arrest of nutrient deprived lung cancer cells. Cytometry 19: 326
21. Wilkins DE, Ng CE, Raaphorst GP (1996) Cisplatin and low dose rate irradiation in cisplatin resistant and sensitive human glioma cells. Int J Radiat Oncol Biol Phys, 36: 105
22. Zolzer F, Streffer C, Pelzer T (1993) Induction of quiescent S-phase cells by irradiation and/or hyperthermia. I. Time and dose dependence. Int J Radiat Biol 63: 69
23. Zolzer F, Streffer C, Pelzer T (1993) Induction of quiescent S-phase cells by irradiation and/or hyperthermia. II. Correlation with colony forming ability. Int J Radiat Biol 63: 77