

## Comparison of Human Tumour Cell Responses to Cisplatin and ZD0473 with and without Irradiation

G.P. RAAPHORST, D.P. YANG, L.F. LI and S. MALONE

*Ottawa Regional Cancer Centre, 503 Smyth Rd., Ottawa, Ontario K1H 1C4, Canada*

**Abstract.** *Three pairs of human tumour cell lines, with one line of each pair resistant to cisplatin, were used to compare the effects of cisplatin and ZD0473 on cellular toxicity and radiosensitization. Whilst all three cell line pairs had one line that was resistant to cisplatin, for ZD0473 the lung tumour HTB56<sub>cp</sub> and cervical carcinoma ME180 cell lines did not express resistance to their HTB56 and SHA counterparts, respectively. Only the ovarian carcinoma line A2780<sub>cp</sub> showed resistance to ZD0473 compared to its counterpart A2780<sub>s</sub>. For radiosensitization both cisplatin and ZD0473 show additive and subadditive effects in the ovarian carcinoma lines, and additive and superadditive effects in the cervical carcinoma and lung tumour cell lines. In fact in the lung tumour cell lines ZD0473 appeared to be a more effective radiosensitizer than cisplatin.*

In a 1969 report in *Nature* by Rosenberg *et al.*, it was indicated that a new class of potent platinum anti-tumour agents had been discovered (1). Since then numerous studies have been done to characterize the effects of such compounds as cisplatin in terms of tumour cell toxicity (2-6). In the course of such studies it was discovered that most cell lines have the capability to develop resistance to cisplatin through a diversity of mechanisms including reduced DNA repair, reduced drug uptake, increased levels of sulphur-containing ligands, increased glutathione transferase and increased metallothionein (note this protein has multiple sulphur ligands) (4-11). In some cell lines more than one resistance mechanism was identified (12,13). Such resistance reduced the effectiveness of cisplatin in killing tumour cells. Cisplatin was also found to be an effective radiosensitizer (14-19) and it was shown that acquired cisplatin resistance could reduce its effect as a radiosensitizer (20,21). In order to overcome these problems platinum compounds were

developed with the objective of overcoming cisplatin resistance (22). One such compound, ZD0473, was developed which has increased steric bulk around the platinum center resulting in reduced reactivity with sulphur ligands such as GSH, thus possibly avoiding this resistance mechanism (13, 22-24). It was shown in experimental models that ZD0374 could be used to circumvent specifically such resistance mechanisms which were observed for cisplatin (25,26).

In this study we set out to compare the effect of cisplatin and the analogue ZD0473 in three pairs of tumour cell lines, each pair having a sensitive and resistant line to cisplatin. The cytotoxic and radiosensitizing effect of each drug was tested and compared to determine if differences exist in the drug resistance expression as well as radiosensitization properties.

### Materials and Methods

The cell lines used in this study were as follows: human ovarian carcinoma A2780<sub>s</sub> derived from an untreated patient (4) and the cisplatin-resistant A2780<sub>cp</sub> line, which was made resistant by stepwise treatment with cisplatin, the HTB56 lung adenocarcinoma line (obtained from ATCC) and the variant HTB56<sub>cp</sub>, which was developed by chronic exposure of HTB56 to cisplatin, and the cervical carcinoma cell lines ME180 cisplatin-resistant and SHA, which were derived from two different patients and were a kind gift from Dr. R.P. Hill.

All cell lines were grown in a mixture of 1:1 DMEM and F-12 medium supplemented with 10% fetal calf serum, 0.1 mM non-essential amino acids, 10 mM sodium bicarbonate and 20 mM HEPES buffer and incubated in a 37°C incubator with 2% CO<sub>2</sub> and 98% air. The cell lines were maintained in exponential growth phase and all experiments were done on exponentially growing cells. The plating efficiencies of the cell lines were A2780<sub>s</sub> and A2780<sub>cp</sub>, 30-50%, the HTB56 and HTB56<sub>cp</sub>, 40 to 60% and the SHA and ME180, 40 to 60%.

For experimental procedures exponentially growing cells were trypsinized, counted and plated into 25-cm<sup>2</sup> tissue culture flasks at numbers required for the experimental procedures. After overnight incubation the cells had attached to the plastic and experimental procedures were started.

Cisplatin was obtained as cisplatin injection (David Bull Canada Inc., Vaudreuil, PQ, Canada) consisting of 1 mg/ml (3.33 mM) cisdiaminedichloroplatinum (II) and 9 mg/ml NaCl, pH adjusted to

*Correspondence to:* Dr. G. Peter Raaphorst, Head, Medical Physics, Ottawa Regional Cancer Centre, 503 Smyth Rd., Ottawa, Ontario K1H 1C4, Canada. Tel: (613) 737-7700 Ext. 6727, Fax: (613) 247-3507, e-mail: graaphorst@orcc.on.ca

**Key Words:** Radiosensitization, cisplatin, ZD0473, human tumour cells.

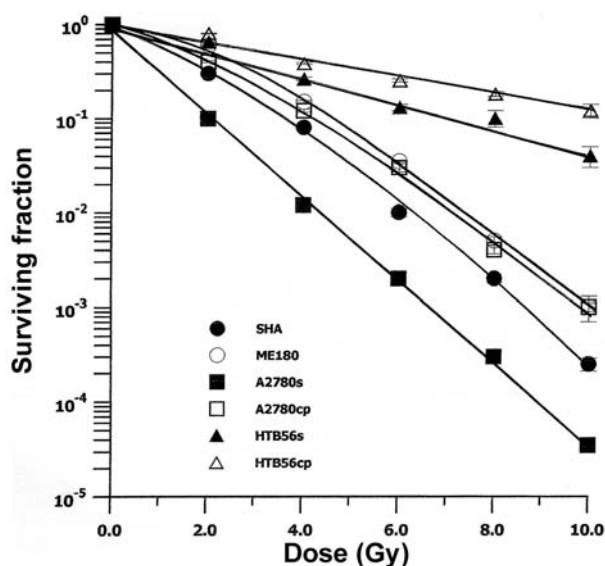


Figure 1. The radiation response for three pairs of cell lines is shown: SHA and ME180, A2780<sub>s</sub> and A2780<sub>cp</sub>, HTB56<sub>s</sub> and HTB56<sub>cp</sub> are human cervical carcinoma, ovarian carcinoma and lung adenocarcinoma cell lines, respectively.

7.3. Cells were treated by adding a measured amount of this solution directly to the culture medium covering the cells. At the end of the exposure period, the medium containing cisplatin was aspirated, and cells were rinsed twice with isotonic citrate saline and new medium was added. In these experiments cells were exposed for 1 hour to cisplatin unless otherwise indicated.

ZD0473 was obtained from Astra Zeneca. The drug was suspended in culture medium at a high concentration and then diluted directly to the experimental culture at the desired concentration.

The irradiation was done using a 250 kV X-ray unit (12.5 mA), with 1.87 mm Al filter running at a dose rate of 168 cGy/min. Since radiation exposure was brief the cells in flasks were removed from the incubator, irradiated at room temperature and then returned to the incubator. For combined treatments irradiation was done 10 minutes after the drug was removed.

After all experimental procedures were completed, the flasks were incubated for 7 to 14 days to allow colony formation for the survival assay. The flasks were then rinsed, stained and the colonies were counted. All experiments were repeated three or more times and the standard error of the mean is shown for the error bars in the figures.

## Results

The response of three pairs of tumour cell lines to radiation is shown in Figure 1. Each pair of tumour cell lines has one cell line that is more resistant to radiation than the other. This difference is the largest for the ovarian carcinoma cell lines A2780<sub>s</sub> and A2780<sub>cp</sub>, next largest for the cervical carcinoma cell line pair SHA and ME180 and the smallest

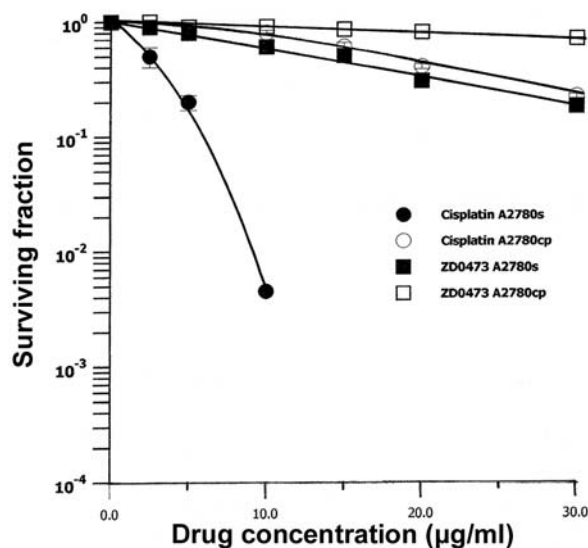


Figure 2. The response of ovarian carcinoma cells to cisplatin and ZD0473 is shown for drug treatments of 1 hour.

for the lung adenocarcinoma HTB56<sub>s</sub> and HTB56<sub>cp</sub>. The radiation survival curves were fitted using the linear quadratic radiation survival curve model (27).

Figures 2-4 show the survival of the three pairs of cell lines to treatment with cisplatin and the ZD0473 a platinum analogue. The results for the ovarian carcinoma cells, presented in Figure 2, show that the A2780<sub>cp</sub> line was more resistant to both cisplatin and ZD0473 than the sensitive line A2780<sub>s</sub>. Both cell lines were more sensitive to cisplatin than ZD0473. In addition, the difference in response to the drugs for the two cell lines was more pronounced for cisplatin than for ZD0473.

In Figure 3 the results for the cervical carcinoma cell lines show that the response for both cell lines was greater to cisplatin than for ZD0473. For cisplatin the SHA cell line was more sensitive than the ME180 cell line, while for ZD0473 this difference was not significant. The data for lung adenocarcinoma, shown in Figure 4, also shows that the responses to cisplatin are greater than to ZD0473 for both cell lines. The differences in responses to drugs for the sensitive HTB56<sub>s</sub> and the resistant HTB56<sub>cp</sub> cell line are large for cisplatin and not significant for ZD0473.

For the lung and ovarian tumour cell lines, the cisplatin-resistant mutant was developed from the parental strain while the SHA and ME180 cervical carcinoma lines were derived from two different human tumours with different cisplatin sensitivity. This difference occurring *in vivo* represents a more natural clinical situation in variation of

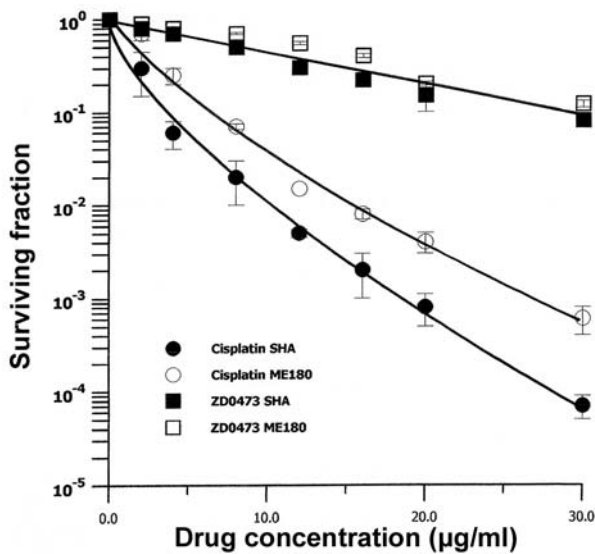


Figure 3. The response of cervical carcinoma cells to cisplatin and ZD0473 is shown for drug treatments of 1 hour.

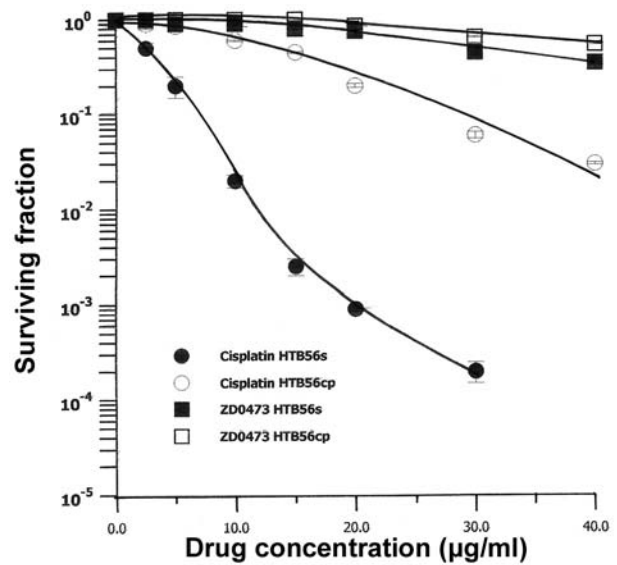


Figure 4. The response of lung adenocarcinoma cells to cisplatin and ZD0473 is shown for drug treatments of 1 hour.

tumour resistance and therefore these cell lines were further tested at higher ZD0473 doses in order to determine if drug resistance observed for cisplatin (Figure 3) at lower survival levels could be overcome. The data in Figure 5 show that, even at high drug concentrations of 10 and 20  $\mu\text{g/ml}$  for exposure times up to seven hours, which reduced survival up to four log orders, the difference in response observed for cisplatin was eliminated by ZD0473.

Because cisplatin is used with radiation therapy, the combined effects of cisplatin and ZD0473 with radiation were also tested in the three cell line pairs. Tables I to III show the results of the three cell line pairs treated with radiation alone or given a 1-hour exposure to drug before irradiation. The survival ratio was calculated by multiplying the survival of the two independent treatments and dividing by the actual survival of the combined treatment. A result greater than one would indicate superadditive effects. For the ovarian carcinoma cells in Table I no values were significantly greater than 1, while for both cisplatin and ZD0473 some values were less than one indicating subadditivity. A radiation dose of 6Gy was chosen in order to have significant radiation cell killing in all cell lines and in order to be off the shoulder region of the radiation survival curve.

For the cervical carcinoma cell line ME180, both cisplatin and ZD0473 showed potential for some superadditivity at several concentrations, while subadditive results were observed in the SHA cell line. In order to explore this further the exposure time of ZD0473 was increased to 2 hours and concentrations up to 40  $\mu\text{g/ml}$  were tested (Figure

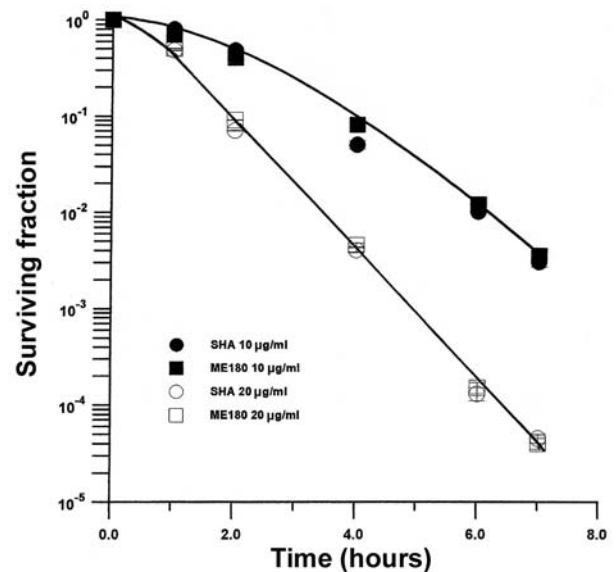


Figure 5. The response of cervical carcinoma cell lines is shown for exposure to 10 and 20  $\mu\text{g/ml}$  ZD0473 for a range of exposure times up to 7 hours.

6). The results show that at high concentrations of ZD0473 (30, 40  $\mu\text{g/ml}$ ) there were superadditive effects for ME180 and for SHA. Note, that the results were normalized to the survival level resulting from radiation alone and thus differences observed in Figure 6 are due to the radiosensitizing effect of the drug.

Table I. Survival ratios for ovarian carcinoma cells.

| Cell Line | Drug/conc. (µg/ml) | Survival Ratio <sup>1</sup> |
|-----------|--------------------|-----------------------------|
| A2780s    | Cisplatin 5        | 0.90 (0.05)                 |
|           | Cisplatin 10       | 1.10 (0.05)                 |
|           | Cisplatin 20       | 0.85 (0.06)                 |
|           | Cisplatin 30       |                             |
| A2780cp   | Cisplatin 5        | 1.03 (0.05)                 |
|           | Cisplatin 10       | 0.91 (0.04)                 |
|           | Cisplatin 20       | 1.0 (0.03)                  |
|           | Cisplatin 30       | 0.85 (0.006)                |
| A2780s    | ZD0473 5           | 1.0 (0.04)                  |
|           | ZD0473 10          | 0.83 (0.05)                 |
|           | ZD0473 20          | 0.75 (0.06)                 |
|           | ZD0473 30          | 0.60 (0.04)                 |
| A2780cp   | ZD0473 5           | 1.0 (0.04)                  |
|           | ZD0473 10          | 1.0 (0.05)                  |
|           | ZD0473 20          | 1.0 (0.06)                  |
|           | ZD0473 30          | 0.8 (0.05)                  |

Survival Ratio =  $\frac{\text{Survival drug} \times \text{Survival 6 Gy}}{\text{Survival combined treatments}}$

Numbers in the brackets represent the standard error of the mean

Table II. Survival ratios for cervical carcinoma cells.

| Cell Line | Drug/conc. (µg/ml) | Survival Ratio <sup>1</sup> |
|-----------|--------------------|-----------------------------|
| SHA       | Cisplatin 5        | 0.90 (0.05)                 |
|           | Cisplatin 10       | 0.88 (0.05)                 |
|           | Cisplatin 20       | 0.12 (0.02)                 |
|           | Cisplatin 30       | 0.15 (0.15)                 |
| ME180     | Cisplatin 5        | 1.70 (0.05)                 |
|           | Cisplatin 10       | 1.40 (0.05)                 |
|           | Cisplatin 20       | 0.80 (0.05)                 |
|           | Cisplatin 30       | 0.66 (0.05)                 |
| SHA       | ZD0473 5           | 0.70 (0.05)                 |
|           | ZD0473 10          | 0.57 (0.05)                 |
|           | ZD0473 20          | 0.55 (0.05)                 |
|           | ZD0473 30          | 0.66 (0.04)                 |
| ME180     | ZD0473 5           | 1.0 (0.04)                  |
|           | ZD0473 10          | 0.70 (0.005)                |
|           | ZD0473 20          | 1.0 (0.06)                  |
|           | ZD0473 30          | 1.25 (0.05)                 |

Survival Ratio =  $\frac{\text{Survival drug} \times \text{Survival 6 Gy}}{\text{Survival combined treatments}}$

Numbers in the brackets represent the standard error of the mean

For the lung tumour cell lines shown in Table III, all the data for cisplatin show subadditivity for both cell lines. For ZD0473 all the results and survival ratio values were around one and thus demonstrated additivity, indicating that ZD0473 was superior to cisplatin in this cell line.

## Discussion

It is well known that cisplatin can be a radiation sensitizer as demonstrated in a number of different tumour cell lines (14-19). One of the difficulties is that in many tumours, resistance develops to cisplatin and this can reduce its effectiveness as a cytotoxin (4-11) as well as its effectiveness in radiosensitization (20,21). The development of cisplatin analogues was attempted in order to try to overcome the resistance observed with cisplatin and the reduced radiation sensitization in resistant cells. The analogue ZD0473 was developed for these reasons and there was indication that cells expressing resistance to cisplatin did not always express resistance to ZD0473. In our experiments we have shown that this was indeed the case. For the cervical carcinoma cell lines which were derived from two different independent tumours, the resistance observed to cisplatin in the ME180 line compared to the SHA line was not observed for ZD0473 when drug treatments were given that lead to comparable toxic effects.

In the lung tumour cell lines resistance was demonstrated to cisplatin and not to ZD0473 by the HTB56<sub>cp</sub> line.

However, in the ovarian carcinoma lines resistance was demonstrated to both cisplatin and ZD0473 by the A2780<sub>cp</sub> line. In our earlier studies we showed that, in the exponentially growing ovarian carcinoma cell line A2780<sub>cp</sub>, there were three mechanisms of resistance expressed, which included reduced uptake, increased GSH and increased DNA polymerase β activity (12) and multiple mechanisms have also been reported by others (28,29). Thus while ZD0473 is developed to have a reduced sensitivity to sulphur ligands it could still be affected by the other two resistance mechanisms.

For radiosensitization the results were more complex. Neither drug produced super-additive results in either the sensitive or resistant ovarian carcinoma cell line. In the cervical carcinoma cell lines, ZD0473 did produce super-additive effects at high concentrations in both cell lines, while cisplatin only demonstrated this at lower concentrations in the ME180 line.

For the two lung tumour cell lines, the results for cisplatin and ZD0473 were different. Cisplatin produced a sub-additive effect with radiation for both cell lines, while ZD0473 produced an additive effect in both lines. Thus, in this cell line pair ZD0473 was superior to cisplatin in terms of radiosensitization.

In summary, it is shown that ZD0473 has good potential as a radiosensitizer in human tumour cells and may be cell-line dependent. We have shown that in some cell lines additive and superadditive effects can be achieved.

Table III. Survival ratios for lung carcinoma cells.

| Cell Line | Drug/conc. (µg/ml) | Survival Ratio <sup>1</sup> |
|-----------|--------------------|-----------------------------|
| HTB56s    | Cisplatin 5        | 0.30 (0.1)                  |
|           | Cisplatin 10       | 0.50 (0.1)                  |
|           | Cisplatin 20       | 0.44 (0.09)                 |
|           | Cisplatin 30       | 0.06 (0.03)                 |
| HTB56cp   | Cisplatin 5        | 0.47 (0.1)                  |
|           | Cisplatin 10       | 0.25 (0.08)                 |
|           | Cisplatin 20       | 0.10 (0.02)                 |
|           | Cisplatin 30       | 0.04 (0.02)                 |
| HTB56s    | ZD0473 5           | 1.0 (0.1)                   |
|           | ZD0473 10          | 1.0 (0.1)                   |
|           | ZD0473 20          | 0.84 (0.08)                 |
|           | ZD0473 30          | 1.0 (0.1)                   |
| HTB56cp   | ZD0473 5           | 1.0 (0.1)                   |
|           | ZD0473 10          | 1.0 (0.09)                  |
|           | ZD0473 20          | 0.94 (0.09)                 |
|           | ZD0473 30          | 1.1 (0.1)                   |

Survival Ratio =  $\frac{\text{Survival drug} \times \text{Survival 6 Gy}}{\text{Survival combined treatments}}$

Numbers in the brackets represent the standard error of the mean

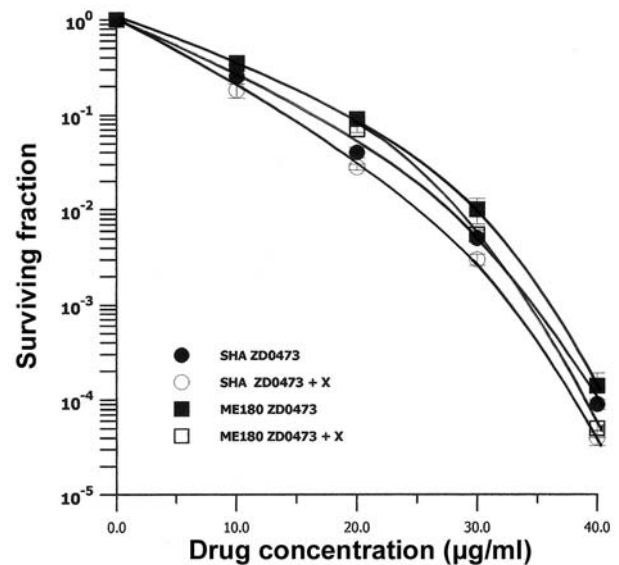


Figure 6. The response of cervical carcinoma cells is shown for exposure to ZD0473 for 2 hours alone or combined with radiation. The radiation dose was 6 Gy and followed 5 minutes after drug treatment.

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