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Preclinical report

Oxaliplatin is active *in vitro* against human melanoma cell lines: comparison with cisplatin and carboplatin

Muneeruddin Q Mohammed[1] and Spyros Retsas[1]
[1]Catherine Griffiths Cancer Research Laboratory, Melanoma Unit, Directorate of Cancer Services and Haematology, Hammersmith Hospitals NHS Trust, Charing Cross Hospital, London W6 8RF, UK.

We have previously confirmed the *in vitro* activity of cisplatin and carboplatin against human melanoma cell lines. Both drugs are important components in the chemotherapy used in our service for advanced metastatic melanoma. In this communication we report the *in vitro* activity of oxaliplatin against human melanoma cell lines in comparison with cisplatin and carboplatin. Oxaliplatin was found to be active against C32 and G361 cell lines with IC_{50} values of 49.48 and 9.07 μM (1 h exposure), 9.47 and 1.30 μM (4 h exposure), and 0.98 and 0.14 μM (24 h exposure), respectively. The cytotoxic activity of oxaliplatin in this *in vitro* system appears to be significantly superior to that of carboplatin. Its activity becomes comparatively closer to that of cisplatin as exposure time increases. Indeed at a 24 h exposure oxaliplatin appears to be significantly more active than cisplatin against the G361 cell line (p=0.0343). Oxaliplatin merits evaluation in the clinic both as a single agent and in combination with other drugs active against melanoma. [© 2000 Lippincott Williams & Wilkins.]

Key words: Activity, carboplatin, cisplatin, *in vitro*, melanoma, oxaliplatin.

Introduction

Cisplatin has been in clinical use for the treatment of a wide range of cancers for nearly 30 years. Its toxic profile has stimulated the development of less toxic analogs, i.e. carboplatin and more recently oxaliplatin. The latter is a third-generation platinum compound, characterized by a 1,2-diaminocyclohexane (DACH) platinum carrier ligand.[1] Its efficacy has already been demonstrated in hitherto refractory neoplasms such as colorectal cancer where it is now used as first-line chemotherapy in combination with 5-fluorouracil.[2]

We have confirmed in this Unit the *in vitro* activity of cisplatin and carboplatin against human melanoma cell lines.[3,4] Both drugs are important components of combination chemotherapy used in our service for advanced metastatic melanoma.[5–7]

In our *in vitro* systems we found that the activity of cisplatin is superior to carboplatin with at least a 4-fold difference in potency.[4] In our experience cisplatin appears to be more active in the clinic than carboplatin, although the latter is better tolerated.[5–7]

We were prompted to investigate oxaliplatin in our *in vitro* systems because of its improved toxicity profile in comparison to cisplatin and carboplatin,[8] its *in vitro* activity against cisplatin-resistant cell lines,[9,10] the relative lack of cross-resistance with the other two drugs,[11] its different mechanism of action to cisplatin,[11] and its additive or partially synergistic activity *in vitro* with the latter.[11] Furthermore, in early clinical studies oxaliplatin has shown some activity against melanoma.[12]

In this communication we report the effect of oxaliplatin in comparison to cisplatin and carboplatin against two human melanoma cell lines.

Material and methods

Cell lines

The melanotic G361 and amelanotic C32 human melanoma cell lines were used in this study as previously described.[13,14] G361and C32 were obtained from the ECACC (Porton Down, UK). Cell lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Labtech, East Sussex, UK), 50 IU/ml penicillin, 2 mM L-glutamine, 50 μg/ml

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Correspondence to S Retsas, Department of Medical Oncology, Melanoma Unit, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK.
Tel: (+44) 20 8746 8295; Fax: (+44) 20 8748 5665;
E-mail: s.retsas@ic.ac.uk

streptomycin (Hyclone, Cramlington, UK), and 2.5 μg/ml amphotericin B (Gibco/BRL, Paisley, UK). Cultures were maintained at 37°C in 5% CO_2 in a humidified atmosphere.

Drugs

Cisplatin (David Bull Laboratories, Warwick, UK), carboplatin (Teva Pharma, Mijdrecht, The Netherlands) and oxaliplatin (Sanofi, Winthrop, UK) were obtained in their clinical formulation, and then diluted to 400 μM in culture medium before each experiment.

Growth inhibitory assay

Growth inhibitory effect of the drugs was determined by the sulforhodamine B (SRB) assay,[15] performed as described previously.[3] Cell lines were harvested in

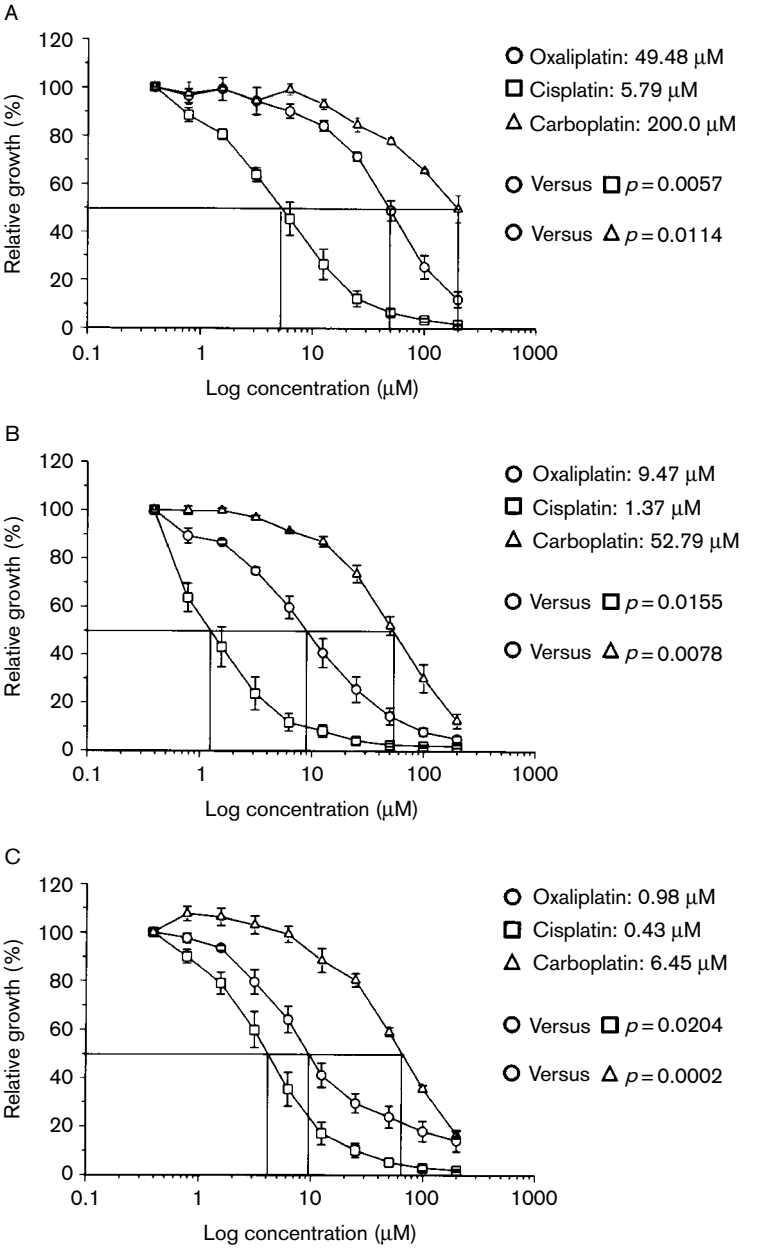

Figure 1. Effect of oxaliplatin, cisplatin and carboplatin against the C32 melanoma cell line: (A) 1 h exposure, (B) 4 h exposure and (C) 24 h exposure. Figures illustrate the mean of three experiments. Mean IC_{50} values for each drug at each exposure time are shown. *p* values were obtained using the Student's *t*-test ($p \leqslant 0.05$ are significant). Vertical error bars indicate standard error of the mean.

0.01% trypsin and 0.004% EDTA solution, resuspended in culture medium, and seeded at 3000 cells/well/100 μl in 96-well microtiter plates. After allowing cells to attach overnight, the cultures were exposed individually to each drug. The drugs were serially diluted 1:2 with the highest concentration being 200 μM for 1 and 4 h, and 20 μM for 24 h exposure times. After drug exposure the cells were washed with 100 μl of PBS and 200 μl of culture media was added to each well. The cultures were then incubated for a further 6 days after the drugs were first added.

Statistical analysis

The differences between the mean IC_{50} values were analyzed with the Student's *t*-test, $p \leqslant 0.05$ was considered to be significant.

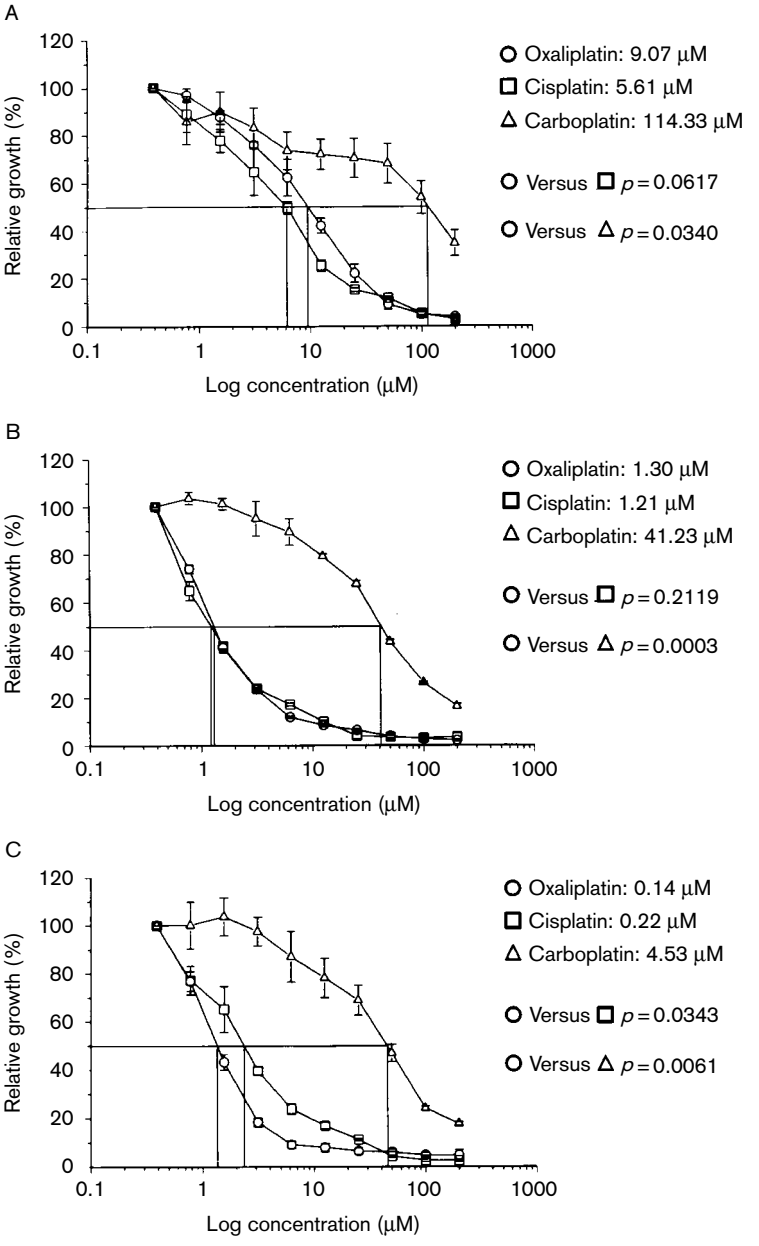

Figure 2. Effect of oxaliplatin, cisplatin and carboplatin against the G361 melanoma cell line: (A) 1 h exposure, (B) 4 h exposure and (C) 24 h exposure. Figures illustrate the mean of three experiments. Mean IC_{50} values for each drug at each exposure time are shown. *p* values were obtained using the Student's *t*-test ($p \leqslant 0.05$ are significant). Vertical error bars indicate standard error of the mean.

Results

The effect of oxaliplatin on the G361 melanoma cell line was greater than that on the C32 cell line. The mean IC_{50} values were 49.48 and 9.07 μM (1 h exposure), 9.47 and 1.30 μM (4 h exposure), and 0.98 and 0.14 μM (24 h exposure) for C32 and G361, respectively (Figures 1 and 2).

In both cell lines and in all experiments irrespective of exposure time oxaliplatin was more effective than carboplatin. On the C32 melanoma cell line the effect of oxaliplatin lies at all exposure times between that of cisplatin (greatest effect) and carboplatin (least effect) (Figure 1). Comparison of the IC_{50} values of oxaliplatin and cisplatin and of oxaliplatin and carboplatin showed that they were significantly different at each exposure time (Figure 1).

On the G361 cell line the effect of oxaliplatin was greater than that of carboplatin and the difference in IC_{50} values at each exposure time was statistically significant (Figure 2).

Oxaliplatin appeared less effective than cisplatin after a 1-h exposure but this did not achieve statistical significance (p=0.0617). After a 4-h exposure the activity of oxaliplatin was similar to cisplatin (p=0.2119). However, after a 24-h exposure oxaliplatin was more active than cisplatin and this difference achieved statistical significance (p=0.0343) (Figure 2).

Oxaliplatin appears to have a time-related effect. As exposure time increases, the effect of oxaliplatin shifts towards the left in the dose–response curves, showing greater cytotoxic effect (Figures 1 and 2), and the mean IC_{50} values become comparatively closer to those of cisplatin and further away from carboplatin (Table 1).

Discussion

Our *in vitro* observations indicate that oxaliplatin has significant cytotoxic activity against human melanoma cell lines. These observations are consistent with an earlier report by Raymond *et al*. who studied the cytotoxicity of oxaliplatin using the human tumor cloning assay.[16]

We have extensively investigated these melanoma cell lines in the past and our current experiments confirm earlier observations of the superior cytotoxicity of cisplatin versus carboplatin.[4] In the present study oxaliplatin appears to be consistently more active than carboplatin but less effective than cisplatin. However, this difference between oxaliplatin and cisplatin is less pronounced in the G361 cell line; indeed on 24-h exposure oxaliplatin appears to be significantly more active than cisplatin. The observed cytotoxicity of oxaliplatin against the G361 melanoma cell line falls within clinically achievable concentrations.[17] It has been suggested that oxaliplatin forms platinum–DNA adducts slowly *in vitro*.[18] This may explain the enhanced cytotoxicity that is observed with prolonged exposure of the cell cultures to the drug.[16]

Although the SRB assay is a useful *in vitro* indicator of the cytotoxic potential of anticancer agents there is clearly a distance between the laboratory and clinical reality. Nevertheless, the concurrence of our results with those reported by Raymond *et al*.[16] indicates that oxaliplatin merits further evaluation in the clinic as single agent and in combination with other active drugs against metastatic melanoma.

Table 1. Factorial difference between the mean IC_{50} values of oxaliplatin in comparison with cisplatin and carboplatin

Cell lines	Exposure times (h)	Cisplatin/ oxaliplatin	Oxaliplatin/ carboplatin
C32	1	8.54	3.03
	4	6.91	5.57
	24	2.29	6.59
G361	1	1.62	12.6
	4	1.07	31.8
	24	0.62	33.1

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