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Preclinical activity of a new platinum analogue, lobaplatin, in cisplatin-sensitive and -resistant human testicular, ovarian, and gastric carcinoma cell lines

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Abstract. Lobaplatin [1,2-diamminomethylcyclobutane-platinum(II) lactate] is a new platinum compound with interesting preclinical activity and apparently no nephro- or neurotoxicity that is currently undergoing clinical phase II studies. Little is known about the cross-resistance between cisplatin and lobaplatin. The activity of this new compound in comparison with cisplatin and carboplatin was evaluated in cisplatin-sensitive and cisplatin-resistant human testicular, gastric, and ovarian carcinoma cell lines using 96 h continuous drug exposure in a sulforhodamine-B assay. In three cisplatin-sensitive testicular carcinoma cell lines, lobaplatin and cisplatin showed comparable antitumor activity. The 50% growth-inhibitory concentrations (IC_{50} values) determined for cisplatin ranged from 0.1 to 0.4 μM, and those found for lobaplatin ranged from 0.25 to 0.5 μM. Carboplatin showed markedly lower cytotoxicity in all cell lines tested. Lobaplatin was not cross-resistant to cisplatin in a 10-fold cisplatin-resistant testicular carcinoma cell line and showed only weak cross-resistance in a 20-fold cisplatin-resistant ovarian carcinoma cell line. In contrast, complete cross-resistance between cisplatin and lobaplatin occurred in two cisplatin-resistant human gastric carcinoma cell lines, which were 3.3- and 9-fold resistant to cisplatin and 3.1- and 6.5-fold resistant to lobaplatin, respectively. Furthermore, lobaplatin showed significant activity against cisplatin-resistant human ovarian and testicular carcinoma xenografts in vivo. These data indicate a high level of activity for lobaplatin at clinically achievable concentrations in drug-sensitive testicular, ovarian, and gastric carcinoma cell lines and a lack of complete cross-resistance to cisplatin. Further clinical development of lobaplatin is clearly warranted.

Introduction

Cisplatin is one of the most frequently used anticancer drugs with documented high activity in a variety of tumor types, including testicular, ovarian, small-cell and non-small-cell lung, head and neck, bladder, and gastric carcinomas [9, 17]. The therapeutic use of cisplatin, however, is limited by severe organ toxicity, predominantly neuro- and nephrotoxicity. Numerous platin analogues have been synthesized and tested in attempts to find platinum compounds with reduced toxicity and/or an extended spectrum of activity. Thus far, only carboplatin has been introduced into the clinic with documented activity in small-cell lung and ovarian cancer and less severe organ toxicity [1, 4].

Lobaplatin is a new cisplatin analogue that has shown promising activity in a variety of preclinical test models, including human lung and stomach cancer xenografts [5]. On the basis of these results, the drug was introduced into clinical phase I trials. During these trials, it was documented that lobaplatin has a favorable spectrum of toxicity, with no nephro- or neurotoxicity being observed thus far. Thrombocytopenia was dose-limiting in these phase I trials [5, 6].

To evaluate the potential role of lobaplatin, we compared its antitumor activity with that of cisplatin in established human gastric, ovarian, and testicular carcinoma cell lines – tumor types that are known to be clinically sensitive to cisplatin. Cisplatin-resistant variants were established from several of these cell lines, and the activity of lobaplatin was tested in the cisplatin-resistant sublines to evaluate the degree of cross-resistance between lobaplatin and cisplatin.

Materials and methods

Drugs and chemicals. Lobaplatin (Fig. 1) was provided by Asta Medica (Frankfurt, Germany), cisplatin was obtained from Medac (Hamburg, Germany), and carboplatin was supplied by Bristol (München, Germany). Culture medium RPMI 1640, fetal calf serum, and trypsin were obtained from Biochrom (Berlin, Germany).

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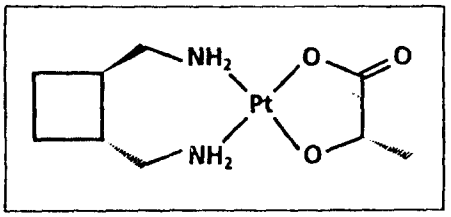

Fig. 1. Structure of 1,2-diamminomethylcyclobutaneplatinum(II) lactate, lobaplatin (D19644)

Cell lines. Lines 2102 EP, H 12.1, and H 32 are human testicular carcinoma cell lines. Line 2102 EP was established by David Bronson and lines H 12.1 and H 32 were established by Jochen Casper. All three lines originated from orchiectomy specimens obtained from nonpretreated patients with nonseminomatous germ-cell cancers. The cisplatin-resistant subline, designated H 12.1 DDP, was developed in our laboratory by sequential exposure of H 12.1 cells to increasing concentrations of cisplatin over a 12-month period. The resulting 10-fold cisplatin resistance remains stable without further drug exposure for at least 3 months.

Lines HM 51 and HM 2 were established in our laboratory from a gastrectomy specimen and from ascitic fluid, respectively, obtained from patients with advanced, nonpretreated adenocarcinoma of the stomach. HM 2 is a moderately differentiated adenocarcinoma and HM 51 is a poorly differentiated anaplastic carcinoma. Both lines grow as continuous monolayers with doubling times of 24–29 h. Two cisplatin-resistant sublines, HM 51 DDP (3.3-fold resistance) and HM 2 DDP (9-fold resistance), were obtained by exposure of the parent lines to increasing concentrations of cisplatin.

Cell line A 2780 was obtained by Rogan et al. [15] from a nonpretreated patient with advanced ovarian carcinoma. The two cisplatin-resistant sublines, designated A 2780 CP2 and A 2780 CP3, were developed by Masuda et al. [10]. They show a stable 10-fold and 20-fold cisplatin resistance, respectively.

All cell lines were maintained as monolayer cultures in RPMI 1640 medium supplemented with 10% fetal calf serum in a humidified incubator containing 5% CO_2 and 95% air at 37° C.

Measurement of cytotoxicity. To assess the cytotoxic effect of cisplatin and lobaplatin, a sulforhodamine-B assay was used as described by Skeehan et al. [16]. In brief, cells were seeded at appropriate densities into 96-well microtiter plates and allowed to attach for 24 h. Then the drugs dissolved in growth medium were added at appropriate concentrations for 96 h. After 96 h drug exposure, the medium was carefully removed and the cells were fixed with 100 µl 10% trichloroacetic acid for at least 1 h. After being washed five times with tap water, the plates were stained with 0.4% sulforhodamine B in 1% acetic acid for 30 min and again washed five times with 1% acetic acid, and the dye was solubilized in 100 µl TRIS base (10 mmol, pH 8.5). The absorbance was read in an automated plate reader at wavelengths of 570 or 595 nm.

Eight separate wells were used for one concentration of drug, and all experiments were performed in triplicate. The concentration that inhibits tumor cell growth by 50% (IC_{50}) was obtained graphically from semilogarithmic dose-response plots. For the cisplatin-resistant sublines, a resistance factor was calculated by dividing the IC_{50} value determined for the resistant cell line by the IC_{50} value found for the corresponding wild-type line.

For the in vivo studies, the cell lines were heterotransplanted by s.c. injection of 1×10^7 cells/mouse into the right flank of 6- to 8-week-old female NMRI nude mice. Tumor measurement were done twice weekly, and the volume was calculated by the formula $v = a \times b^2$, where a is the largest diameter and b is the diameter perpendicular to a. When the tumors had reached an average volume of 1–1.5 cm^3, the mice were divided into groups of 5–8 animals and treatment was initiated. Cisplatin was given at a dose of 4 mg/kg daily on days 1, 3, and 5, and lobaplatin was given at 12 mg/kg daily on days 1, 3, and 5. These doses are equitoxic [approximately corresponding to the doses lethal to 10% of the animals (LD_{10})] as determined in a separate set of experiments using non-tumor-bearing nude mice. The drugs were given by i.p. injection in a volume of 2 ml 0.9% NaCl. Control animals received saline only.

Results

Testicular cancer cell lines

In the three cisplatin-sensitive testicular carcinoma cell lines, cisplatin and lobaplatin showed comparable cytotoxicity, whereas considerably higher concentrations of carboplatin were required (Table 1). However, lobaplatin was significantly more active than cisplatin or carboplatin in the 10-fold cisplatin-resistant testicular carcinoma cell line H 12.1 DDP, indicating a lack of cross-resistance.

Gastric carcinoma cell lines

The two gastric carcinoma cell lines HM 51 and HM 2 required comparable concentrations of cisplatin or lobaplatin for inhibition of cell growth by 50%. The IC_{50} values were 0.3 and 0.9 µ*M*, respectively, for cisplatin and

Table 1. IC_{50} values for cisplatin, carboplatin and lobaplatin

Cell line	Cisplatin (µ*M*)	R_f[a]	Carboplatin (µ*M*)	R_f[a]	Lobaplatin (µ*M*)	R_f[a]
2101 EP	0.16	–	2.10	–	0.25	–
H 32	0.40	–	5.20	–	0.50	–
H 12.1	0.10	–	8.50	–	0.30	–
H 12.1 DDP	1.00	10	65.00	7.6	0.40	1.3
A 2780	0.20	–	6.00	–	0.10	–
A 2780 CP2	3.00	15	52.5	8.7	1.00	10
A 2780 CP3	4.00	20	71.00	11.8	0.50	5
HM 2	0.90	–	ND	–	0.50	–
HM 2 DDP	8.00	9	ND	–	3.20	6.5
HM 51	0.30	–	ND	–	0.50	–
HM 51 DDP	1.00	3.3	ND	–	1.60	3.2

ND, Not determined

[a] R_f (resistance factor) = IC_{50} of the resistant line divided by IC_{50} of the wild-type line

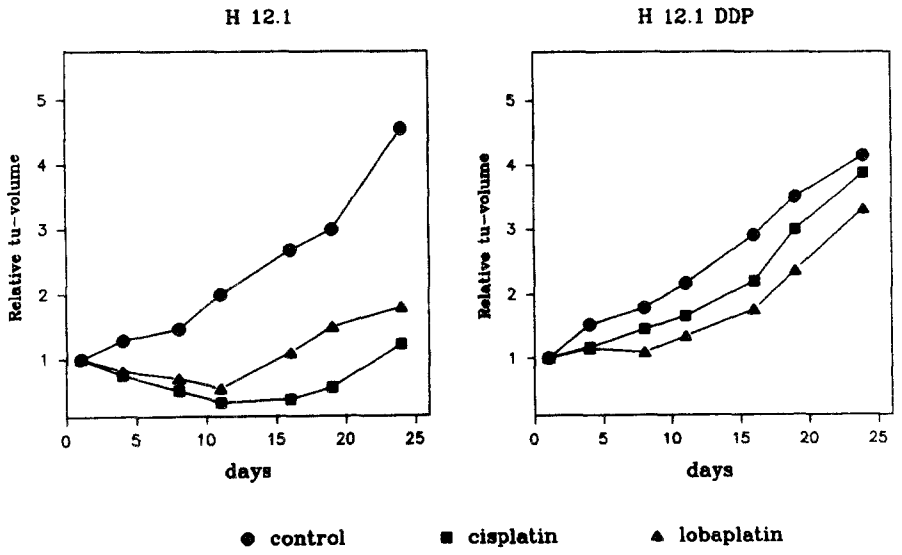

Fig. 2. Antitumor activity of cisplatin and lobaplatin against xenografts from the cisplatin-sensitive testicular cancer cell line H 12.1 and its cisplatin-resistant subline H 12.1 DDP. Both drugs were given to mice at equitoxic concentrations (approx. LD_{10}) by i. p. injection

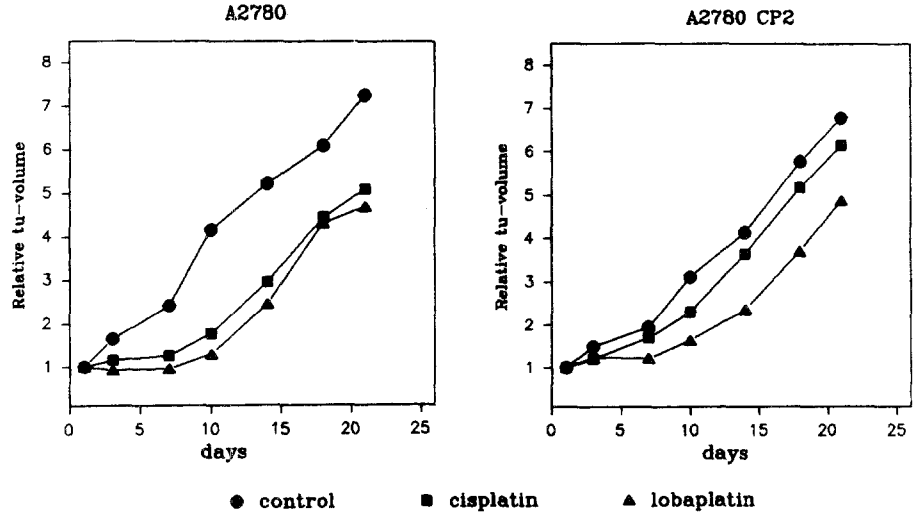

Fig. 3. Antitumor activity of cisplatin and lobaplatin against xenografts from the cisplatin-sensitive ovarian cancer cell line A 2780 and its cisplatin-resistant subline A 2780 CP2. Both drugs were given to mice at equitoxic concentrations (approx. LD_{10}) by i. p. injection

0.9 and 0.5 μ*M*, respectively, for lobaplatin (Table 1). Two cisplatin-resistant sublines, HM 51 DDP and HM 2 DDP, which were 3.3-fold and 9-fold resistant to cisplatin, respectively, as compared with the corresponding wild-type lines, showed almost complete cross-resistance to lobaplatin.

Ovarian carcinoma cell lines

The results obtained for the ovarian carcinoma cell line A 2780 and its two cisplatin-resistant sublines are listed in Table 1. The IC_{50} values for cisplatin and lobaplatin in the wild-type cell line were comparable. However, lobaplatin remained active in the highly cisplatin-resistant line A 2780 CP3 again indicating a lack of complete cross-resistance between these two platinum analogues. Carboplatin was less active than either cisplatin or lobaplatin in all three cell lines (Table 1).

In vivo studies

Cisplatin showed superior antitumor activity as compared with lobaplatin in cisplatin-sensitive xenografts obtained from cell line H 12.1 (Fig. 2). However, in the cisplatin-resistant xenografts originating from line H 12.1 DDP, lobaplatin exhibited antitumor activity stronger than that of cisplatin, thus confirming in vivo the lack of cross-resistance seen in the in vitro experiments. Comparable potency was seen for cisplatin and lobaplatin in A 2780 xenografts, whereas lobaplatin was again slightly more active than cisplatin in cisplatin-resistant A 2780 CP2 xenografts (Fig. 3).

Discussion

The platinum-based compounds cisplatin and carboplatin belong to the most frequently used anticancer drugs in

clinical practice. Cisplatin has demonstrated high activity in several tumor types, including testicular, ovarian, lung, head and neck, esophageal, and gastric carcinomas. Its use, however, is limited by sometimes severe organ toxicity, especially neuro- and nephrotoxicity [9, 17]. Carboplatin shows a favorable spectrum of toxicity, with reversible myelosuppression being the dose-limiting toxicity, whereas significant organ toxicity is observed only after the administration of very high doses of this compound. Carboplatin seems to have comparable activity in ovarian cancer but has been shown to be less active than cisplatin in several other tumors such as gastrointestinal and urothelial cancers [1, 11, 14].

Numerous platinum-based compounds have been synthesized in an attempt to find new platinum analogue that either produce less severe organ toxicity than cisplatin or show a different pattern of activity and, ideally, no cross-resistance to the parent compound. Lobaplatin has been developed for further clinical testing on the basis of its high activity in several preclinical models, including human lung, breast, and gastric carcinoma xenografts [5], and its lack of nephrotoxicity in animal studies [2]. Little is known about the mechanisms of action and the cross-resistance pattern of this new compound. In our in vitro studies, we demonstrated a high degree of activity for lobaplatin in testicular, ovarian, and gastric carcinoma cell lines. The IC_{50} values determined for this drug in all wild-type lines ranged from 0.1 to 0.9 μM. Recent phase I trials have shown that the pleak plasma concentration of lobaplatin after bolus application of 50 mg/m^2 is in excess of 10 μM [13]. Therefore, lobaplatin shows cytotoxic potency in a range of concentrations that seem to be readily achievable in the clinic. Furthermore, all phase I trials have demonstrated that lobaplatin does not produce any nephro- or neurotoxicity. Myelosuppression, especially thrombocytopenia, is dose-limiting [5, 6].

It is noteworthy that lobaplatin seems to lack complete cross-resistance to cisplatin. Lobaplatin showed high activity not only in the cisplatin-sensitive testicular carcinoma cell lines but also in the cisplatin-resistant variant H 12.1 DDP in vitro. The lack of cross-resistance was confirmed in vivo using both drugs at equitoxic concentrations. This panel of testicular carcinoma cell lines has been used to evaluate numerous other anticancer drugs, and the results show good correlation with those obtained in the clinical setting [8]. It is noteworthy that carboplatin was identified to have significantly lower activity than cisplatin in non-seminomatous testicular cancer in this model – a result that has since been confirmed by a randomized clinical trial [3]. An apparent lack of cross-resistance, at least in testicular cancers, has also recently been demonstrated by Meijer and co-workers [12] in the cell line Tera CP. Comparable with the results we obtained in gastric carcinoma cell lines, they found complete cross-resistance between cisplatin and lobaplatin in the small-cell lung carcinoma line GLC4. Apparently, specific mechanisms of cisplatin resistance seem to be operating in the testicular cancer cell lines that do not confer resistance to lobaplatin. The exact mechanisms of this resistance remain to be elucidated.

Lobaplatin also showed very promising activity in the ovarian carcinoma cell lines and again did not appear to be completely cross-resistant to cisplatin. Several mechanisms of resistance to cisplatin, including impaired uptake, increased detoxification by the glutathione pathway, and increased DNA-repair capacity, have been described [7, 10]. For the cisplatin-resistant ovarian carcinoma cell lines A 2780 CP2 and CP3, detoxification by the glutathione pathway and increased removal of DNA adducts play an important role in the resistance to cisplatin [7, 10], whereas the exact mechanisms that are operative in the other cell lines used in the current studies have not been fully explored. Thus, it cannot be assessed as to which of the known mechanisms of resistance to cisplatin will also confer resistance to lobaplatin. However, it might be clinically important that some tumors that become resistant to cisplatin may nonetheless respond to lobaplatin.

In conclusion, considering all preclinical and early clinical findings together, lobaplatin appears to be a highly interesting new drug with a high degree of activity in preclinical models, a lack of complete cross-resistance to cisplatin, and a favorable spectrum of toxicity. Its further clinical development is clearly warranted.

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