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Drug interactions of 5-fluorouracil with either cisplatin or lobaplatin—a new, clinically active platinum analog in established human cancer cell lines

A Harstrick, U Vanhoefer, A Heidemann, H Druyen, H Wilke and S Seeber
Department of Internal Medicine, West German Cancer Center, Hufelandstraße 55, 45122 Essen, Germany. Tel: (+49) 201 7233100; Fax: (+49) 201 7235924.

Lobaplatin [1,2-diaminomethylcyclobutane platinum(II)-lactate] is a new platinum compound which appears to possess incomplete cross-resistance to cisplatin and might have a favorable pattern of side effects. Since lobaplatin has activity in esophageal cancer, combination protocols with 5-fluorouracil (5-FU) are evaluated. In order to assess the mode of action of lobaplatin when combined with 5-FU, two human cancer cell lines were treated with various combinations of 5-FU given for either 2 or 24 h and lobaplatin. Drug interactions were evaluated by isobologram analysis. Lobaplatin showed basically the same interaction pattern when combined with 5-FU as cisplatin. The combination of either platinum analog with a 24 h exposure to 5-FU was superior to a short-term 5-FU exposure. Furthermore, when 5-FU was given for 24 h, no additional effect of folinic acid was seen. From these data we conclude that cisplatin and lobaplatin show similar interactions with 5-FU. Protracted infusion schedules of 5-FU appear to be more active than bolus application.

Key words: 5-Fluorouracil, cisplatin, lobaplatin.

Introduction

Cisplatin is one of the most frequently used antineoplastic drugs in clinical oncology. The drug has a wide range of activity including germ cell and ovarian cancer, lung cancer, bladder and gastric cancer, and squamous cell carcinoma of the esophagus and head and neck.[1] Furthermore, preclinical and clinical studies indicate that cisplatin and 5-fluorouracil (5-FU) can act synergistically against tumor cells.[2–5] However, the administration of cisplatin is accompanied by several adverse side effects, including nephro- and neurotoxicity.

Lobaplatin [1,2-diaminomethylcyclobutane platinum(II)-lactate] is a new platinum compound currently undergoing clinical evaluation in phase II and phase III studies. Lobaplatin has demonstrated antitumor activity in a variety of experimental tumor models, and showed clinical activity in patients with advanced ovarian and esophageal cancer.[6,7] Preclinical data indicate that lobaplatin seems to possess incomplete cross-resistance to cisplatin and responses to lobaplatin have been demonstrated in patients with ovarian cancer after failing cisplatin-based therapy.[6,8] Furthermore, lobaplatin shows a different pattern of side effects compared to cisplatin. The drug has no nephro- or neurotoxicity and can be given as rapid i.v. injection without the requirement of hyperhydration. The dose-limiting toxicities of lobaplatin are myelosuppression, especially cumulative thrombocytopenia.[9,10]

Results of a recent study demonstrated that lobaplatin produced a 28% response rate in patients with metastatic squamous cell carcinoma of the esophagus.[7] To build on these results, a clinical phase III trial comparing a combination of lobaplatin and 5-FU to standard cisplatin/5-FU has been initiated.

To further elucidate the differences between lobaplatin and cisplatin in terms of drug interactions, we performed *in vitro* investigations of both platinum compounds combined with various schedules of 5-FU in established human cancer cell lines. These investigations might serve as a basis for a rational design of clinical combination protocols, especially for patients with platinum sensitive tumors like esophageal cancer, and head and neck carcinoma.

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Correspondence to A Harstrick

Materials and methods

Drugs and chemicals

Lobaplatin (D 19644) was provided by ASTA Medica (Frankfurt, Germany). Cisplatin was obtained from Medac (Hamburg, Germany) and 5-FU was obtained from Sigma (Deisenhofen, Germany), culture medium RPMI 1640, fetal calf serum and trypsin were obtained from Biochrom (Berlin, Germany).

Cell lines

Two established human cancer cell lines were used. Cell line H460, a cell line obtained from a patient with non-small cell lung cancer, was obtained from the ATCC (HTB 177; Rockville, MA). Cell line A2780 was established from a patient with non-pretreated ovarian carcinoma and was provided by Dr YM Rustum (Roswell Park Cancer Center, Buffalo, NY).

Both cell lines were grown in RPMI 1640 growth medium supplemented with 10% fetal calf serum in an atmosphere of 5% CO_2 in air.

Evaluation of cytotoxicity

Cytotoxicity was assessed by the sulforhodamine B assay as described by Skehan *et al.*[11] In brief, cells were seeded at appropriate densities into 96-well microtiter plates and allowed to attach for 24 h. The schedules of drug incubation are detailed below. After drug exposure, the cells were incubated with drug-free medium for additional 96 h. The medium was carefully removed and the cells were fixed with 100 μl 10% trichloroacetic acid for at least 1 h. Staining with 0.4% sulforhodamine B in 1% acetic acid was performed as originally described. The absorbance was read in an automatic plate reader (Dynatech, Denkendorf, Germany) using at a wavelength of 570 nm. Each experiment was repeated three times, and for each experiment and drug concentration eight wells were used. From the data, mean values and standard deviations were calculated and statistical analysis was done by a *t*-test.

Drug interaction studies

To evaluate the drug interactions, the classical isobologram methodology as described by Berenbaum *et al.*[12] was used. The interactions were evaluated at the IC_{50}.

Cisplatin or lobaplatin were always given for 2 h; the plates were then washed and incubated with various concentrations of 5-FU either for 2 or 24 h.

To assess the additional influence of folinic acid on the combination of either cisplatin or lobaplatin with 5-FU, a different experimental approach was used. In these experiments cells were preincubated with either 25 or 50% of the individual IC_{50} for cisplatin or lobaplatin for 2 h, washed and then exposed to 5-FU for 2 or 24 h. Folinic acid was added at a fixed concentration of 20 μM for either 2 or 24 h simultaneously with 5-FU.

Results

The cell lines A2780 and H460 showed differences in their sensitivity to cisplatin, lobaplatin and the two different schedules of 5-FU. In general, H460 cells were more drug sensitive with the exception of their response to lobaplatin, which was more active in A2780 cells. The IC_{50} values for both cell lines are summarized in Table 1.

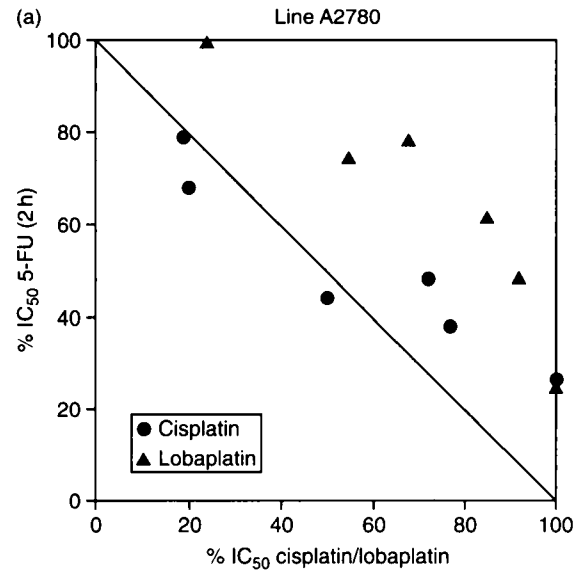
The interaction studies using classical isobologram analysis are shown in Figures 1 and 2. In A2780 cells, cisplatin and a short-term exposure to 5-FU (2 h) were found to be additive whereas the combination of lobaplatin and 5-FU given for 2 h appeared to be antagonistic. However, in cell line H460, both platinum compounds showed antagonistic interactions when combined with a short-term exposure to 5-FU.

A different pattern of drug interactions was seen when the platinum compounds were combined with a protracted exposure to 5-FU (Fig. 2). In both cell lines cisplatin as well as lobaplatin showed additive cytotoxic activity when combined with a 24 h exposure to 5-FU.

In a separate set of experiments, the effect of

Table 1. Concentrations that inhibited cell growth by 50% (IC_{50}) compared to untreated controls (each value represents the mean ± SD of at least four separate experiments)

	H460 (μM)	A2780 (μM)
Cisplatin (2 h)	5.0 ± 0.7	7.5 ± 1.1
Lobaplatin (2 h)	7.3 ± 0.8	6.3 ± 0.5
5-FU (2 h)	21.5 ± 4.7	62.2 ± 5.8
5-FU (24 h)	2.7 ± 0.5	4.8 ± 0.9



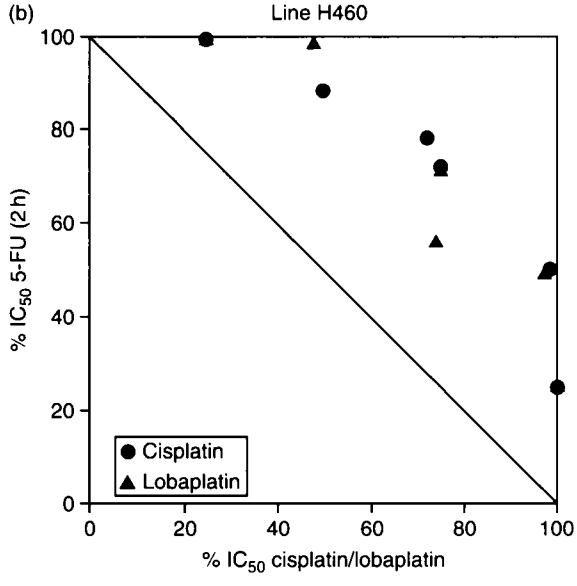
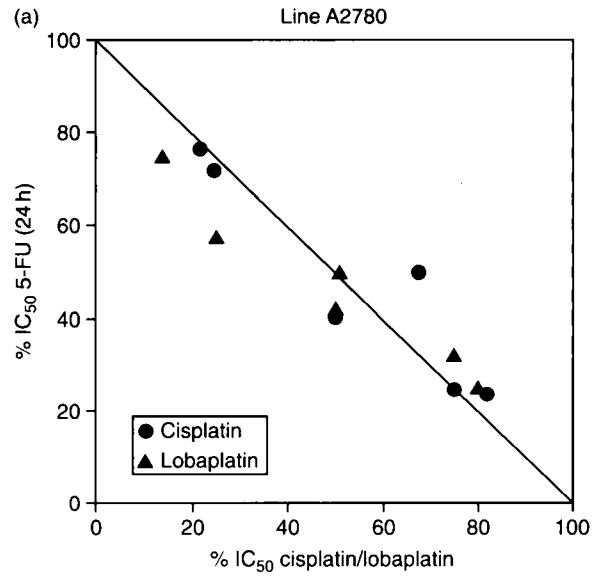


Figure 1. Isobologram analysis of the combination of cisplatin or lobaplatin with a short-term exposure (2 h) to 5-FU in A2780 (a) or H460 (b) cells. Cisplatin or lobaplatin were added for 2 h, the plates were washed and 5-FU was added for 2 h.



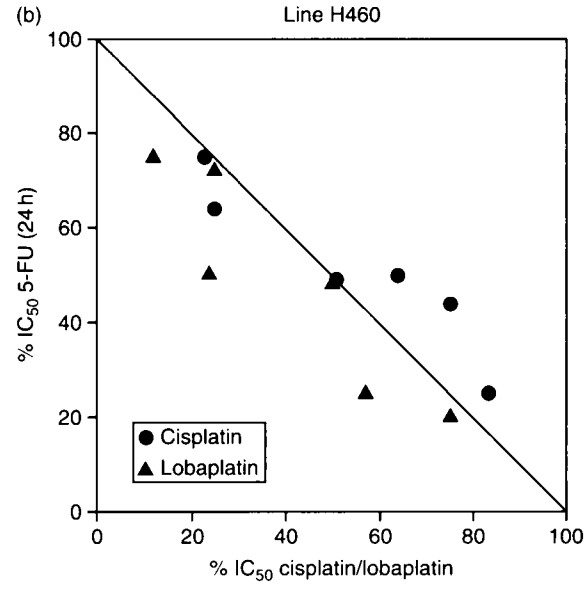


Figure 2. Isobologram analysis of the combination of cisplatin or lobaplatin with a long-term exposure (24 h) to 5-FU in A2780 (a) or H460 (b) cells. Cisplatin or lobaplatin were added for 2 h, the plates were washed and 5-FU was added for 24 h.

folinic acid (20 μM) on the activity of the combination of a platinum compound with 5-FU was investigated. For these experiments cells were incubated with a fixed concentration of either cisplatin or lobaplatin for 2 h, washed and then exposed to various concentrations of 5-FU either with or without folinic acid (20 μM). Results are summarized in Table 2. Folinic acid had no effect on the cytotoxicity of cisplatin/5-FU or lobaplatin/5-FU combinations given in any schedule in cell line A2780. In contrast, in H460 cells the addition of folinic acid significantly increased the cytotoxicity of those combinations in which 5-FU was applied for 2 h. No effect, however, was seen on the schedules using a protracted 24 h exposure to 5-FU. As for the combination without folinic acid, there were no

Table 2. IC_{50} of 5-FU when combined with cisplatin or lobaplatin

	IC_{50} values (μM)	
	A2780	H460
25% IC_{50} lobaplatin +		
5-FU (2 h)	43.0 ± 6.5	45.8 ± 10.6
5-FU/FA (2 h)	43.3 ± 12.1	32.6 ± 6.8[a]
5-FU (24 h)	3.7 ± 1.7	2.2 ± 0.6
5-FU/FA (24 h)	4.0 ± 1.9	2.2 ± 0.9
25% IC_{50} cisplatin +		
5-FU (2 h)	38.7 ± 7.2	52.7 ± 11.1
5-FU/FA (2 h)	37.0 ± 10.1	41.5 ± 12.4
5-FU (24 h)	2.6 ± 0.3	3.3 ± 1.4
5-FU/FA (24 h)	2.2 ± 0.4	2.1 ± 0.6
50% IC_{50} lobaplatin +		
5-FU (2 h)	30.6 ± 7.1	29.6 ± 6.1
5-FU/FA (2 h)	32.1 ± 6.5	20.8 ± 5.9[a]
5-FU (24 h)	2.7 ± 1.3	1.2 ± 0.5
5-FU/FA (24 h)	2.9 ± 0.8	0.9 ± 0.6
50% IC_{50} cisplatin +		
5-FU (2 h)	28.1 ± 7.4	43.0 ± 7.2
5-FU/FA (2 h)	31.0 ± 6.5	34.1 ± 8.1[a]
5-FU (24 h)	1.6 ± 0.3	2.7 ± 1.3
5-FU/FA (24 h)	1.5 ± 0.4	1.8 ± 0.7

[a]Denotes statistically significant difference between schedule without and with folinic acid ($p < 0.05$). Cells were exposed to a fixed concentration of cisplatin or lobaplatin (25 or 50% of individual IC_{50}), washed and incubated with 5-FU for 2 or 24 h either with or without folinic acid (FA; 20 μM). Data represent the mean ± SD of three separate experiments.

differences in terms of drug interaction between cisplatin and lobaplatin.

Discussion

The combination of 5-FU and cisplatin has demonstrated significant activity in patients with advanced squamous cell carcinoma of the esophagus and head and neck region.[4,5] Lobaplatin is a new platinum analog with documented clinical efficacy in esophageal cancer and has a favorable pattern of side effects. Therefore attempts are made to replace cisplatin by lobaplatin in combination protocols for esophageal and head and neck cancer. However, since lobaplatin and cisplatin display incomplete cross-resistance and might possess differences in the activating pathways or modes of cytotoxic action,[8] we investigated the *in vitro* interactions of lobaplatin when given in combination with various schedules of 5-FU to develop guidelines for the design of clinical protocols. For these investigations, two different schedules of 5-FU which are frequently used clinically were employed. Recent preclinical and clinical findings indicate that the mode of cytotoxic action of 5-FU might change depending on the way of administration. If cells are exposed to 5-FU for a short period of time, the main mechanism of resistance appears to be RNA mediated.[13] However, cells with acquired resistance to protracted exposure times to 5-FU display resistance by means of changes in the thymidylate synthase pathways.[14] Furthermore, for colorectal cancer and gastric cancer it has been demonstrated that a protracted infusion schedule of 5-FU, given either as 24 h infusion or as infusion over several weeks, can overcome clinical resistance to bolus 5-FU regimens.[15-17] Therefore it is possible that interactions with other cytotoxic drugs might also differ depending on the schedule of 5-FU used.

The results of the present study suggest that there are no differences between lobaplatin and cisplatin in the way they interact with 5-FU when isobologram methodology is employed. For both platinum analogs, superior cytotoxic activity was seen when they were combined with a protracted exposure to 5-FU.

Biochemical modulation of the activity of 5-FU by folinic acid was only seen in H460 cells and was schedule dependent.

Folinic acid had no effect on the activity of the platinum/5-FU combination when 5-FU was given for 24 h. In contrast, when a short exposure of 5-FU was used, folinic acid significantly increased the activity of the combination compared to the non folinic acid containing regimen. These findings together with recent clinical data in colorectal cancer, demonstrating significant antitumor activity of 5-FU given as high dose 24 h infusion or as protracted infusion for several weeks[18] without folinic acid, raise the question about the role of folinic acid in combination with protracted 5-FU infusions.

In conclusion, our data have shown that there are no differences in terms of drug interactions between cisplatin and lobaplatin when combined with 5-FU *in vitro*. Since the combination of lobaplatin with short-term exposure to 5-FU produced antagonistic interactions and might also possess overlapping dose-limiting toxicities, e.g. myelosuppression, we advocate to combine lobaplatin with a protracted infusion of 5-FU for clinical protocols. If 5-FU is given for 24 h, the addition of folinic acid does not seem to increase the cytotoxicity of the combination. These results will be evaluated clinically in an ongoing phase III protocol, comparing the activity of lobaplatin to cisplatin in combination with pro-

tracted infusion of 5-FU in patients with advanced esophageal cancer.

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