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Cycloplatam: A Novel Platinum Compound Exhibiting a Different Spectrum of Anti-tumour Activity to Cisplatin

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Cycloplatam is a novel platinum compound which has shown anti-tumour activity in murine tumour models. In this study, cycloplatam was found to have anti-tumour activity *in vitro* and *in vivo* in human tumour models. In 15 cell lines (mainly ovarian), cycloplatam showed similar cytotoxicity as cisplatin, using the sulphorhodamine B assay. Determination of the resistance factor (IC_{50} of cisplatin-resistant divided by IC_{50} of parental cell line) clearly showed lower values for cycloplatam than for cisplatin. In the parental ovarian cell line CH1 and the cisplatin-resistant CH1cisR model, we observed no cross-resistance of cycloplatam and cisplatin. The *in vitro* anti-tumour activity was confirmed in human tumour xenografts using the clonogenic assay. Mean IC_{70} values of cycloplatam were 0.54 μg/ml (1.25 μM) and of cisplatin 0.42 μg/ml (1.4 μM), respectively. In the murine subcutaneously implanted ADJ/PC6 plasmacytoma *in vivo* cycloplatam showed less activity than cisplatin, with a 2-fold smaller therapeutic index than cisplatin. In ovarian cancer xenografts cycloplatam was less active than cisplatin. However, anti-tumour activity of cycloplatam in lung cancer xenografts was quite different from cisplatin. In LXFS 538, a model moderately sensitive to cisplatin, a partial remission was observed, but in LXFL 529, a cisplatin-sensitive model, cycloplatam was inactive, cycloplatam thus demonstrating a different spectrum of anti-tumour activity. Based on these results, further preclinical investigations with other tumours, such as cisplatin-sensitive and -resistant gastric cancer models, are warranted with cycloplatam.

Key words: cycloplatam, cisplatin, cytotoxicity, ovarian cancer xenografts, lung cancer xenografts
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INTRODUCTION

CISPLATIN IS one of the most active compounds in the clinical treatment of cancer. Alone or in combination, cisplatin is now the mainstay of treatment for testicular and ovarian cancer [1–3]. However, a number of tumours are unresponsive and others develop resistance after an initial response [4]. The unfavourable toxicity profile (nephrotoxicity and neurotoxicity) resulted in the development of second generation platinum compounds with the intention of circumventing cisplatin resistance and to reduce toxicity [5, 6]. However, only one of the second generation platinum compounds, carboplatin, has received clinical acceptance. Carboplatin exhibits reduced side-effects (dose-limiting factor is myelosuppression), but the spectrum of clinical activity is similar to that of cisplatin [7–9].

The main goal in developing further platinum-based complexes is to circumvent cisplatin resistance or to reach a low level of cross-resistance to cisplatin. Cycloplatam (Ammine-cyclopentylamine-S-(-)-malatoplatinum) [10, 11] is a new platinum compound discovered by the N.S. Kurnakow Institute of General and Inorganic Chemistry, Russian Academy of Science, Moscow. Cycloplatam exists in equilibrium between two isomeric forms (Figure 1). In our hands, cycloplatam showed, *in vitro*, a higher chemical reactivity than carboplatin which could be effected through the shifting hydroxyl group. However, the mechanism of action and the importance of the isomeric forms are still unknown.

The anti-tumour activity of cycloplatam was described by the Cancer Research Centre of the Russian Academy of Medical Science (Moscow) in murine solid tumour models and leukaemias [10]. Cycloplatam showed less toxicity *in vivo* than cisplatin and higher anti-tumour activity *in vivo* than carboplatin [10, 12]. However, these promising preclinical data have been obtained in murine tumour models. Since human tumour models are known to be more predictive of the clinical situation in patients, the preclinical activity of cycloplatam needs to be established in human tumour systems. In our present study, we describe the *in vitro* and *in vivo* anti-tumour activity of cycloplatam compared with cisplatin in human tumour models.

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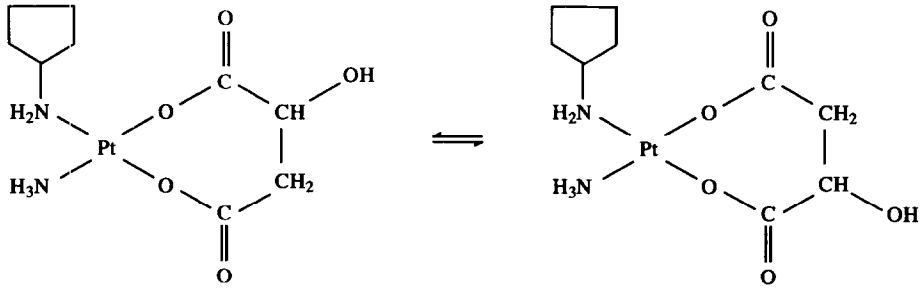

Figure 1. Chemical structure of cycloplatam.

MATERIALS AND METHODS

Drugs

Cycloplatam (see Figure 1 for structure) was supplied by the Cancer Research Centre in Moscow via the EORTC New Drug Development Office. For *in vitro* studies and administration to animals, cycloplatam was dissolved in water immediately before use. For all studies, cisplatin was used as the clinical formulation obtained from Bristol-Arzneimittel GmbH (Munich, Germany).

In vitro *cytotoxicity studies*

The cytotoxicity of cycloplatam was assessed in human tumour cell lines and xenografts using the sulphorhodamine B assay [13, 14] and the clonogenic assay [15, 16]. Both assay systems have been described extensively elsewhere [14, 16]. Briefly, the sulphorhodamine B assay involves plating of the cells, preincubation for 24 h, followed by 96-h continuous drug exposure over a broad concentration range. The effect of drug exposure was determined at the end of the appropriate exposure period and two evaluation parameters were used: drug concentration that inhibits growth by 50% (IC_{50}) and resistance factor, which is the ratio of IC_{50} value in the resistant line (IC_{50} cisR line) and IC_{50} value of the drug in the parent line (IC_{50} parent line). Six "parent" human ovarian carcinoma cell lines were used. SKOV-3 [17] was obtained from the American Type Culture Collection. A2780 was kindly provided by Dr T. Hamilton (Fox Chase Cancer Center, Philadelphia, Pennsylvania, U.S.A.). Establishment details and biological properties of the remaining four lines (HX/62, PXN/94, CH1 and 41M) have been described previously [18].

In addition, six pairs of human tumour cell lines (parent and derived subline with acquired resistance to cisplatin) were used: 41M/41McisR; CH1/CH1cisR; A2780/A2780cisR; OVCAR-3/OVCAR-3cisR (all ovarian); GCT27/GCT27cisR (testicular non-seminomatous germ cell) and HX155/HX155cisR (cervical carcinoma). The parent 41M, A2780, GCT27 and HX/155 lines were all derived from previously untreated patients. Establishment details of 41M/41McisR, CH1/CH1cisR, A2780/A2780cisR, GCT27/GCT27cisR and HX155 have been described previously [14, 18–20]. The induction of drug resistance to cisplatin has also been described previously [14, 19]. Briefly, cells were exposed to increasing concentrations of drug (starting at an approximately 10% inhibitory concentration) over a 12–18 month period. Typically, cells were exposed three times to each concentration, after which the concentration was doubled. Exposure was continuous over 3 days; the drug was then removed, and the cells were exposed again when normal growth had resumed.

All lines grew as monolayers in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 50 μg/ml gentamicin, 2.5 μg/ml amphotericin B, 2 mM L-glutamine, 10 μg/ml insulin and 0.5 μg/ml hydrocortisone in 10% CO_2/90% air.

Further characterisation of the *in vitro* cytotoxicity profile of cycloplatam was carried out in human solid tumour xenografts using the clonogenic assay with continuous drug exposure [21, 22]. Briefly, solid human tumour xenografts were mechanically disaggregated and subsequently incubated with an enzyme cocktail consisting of 0.05% collagenase, 0.07% DNAse and 0.1% hyaluronidase in RPMI 1640 at 37°C for 30 min. The cells were washed twice and passed through sieves of 200 and 50 μm mesh size. The percentage of viable cells was determined in a haemacytometer using trypan blue exclusion. The tumour cell suspension was plated into 24-multiwell plates over a bottom layer consisting of 0.2 ml Iscoves's modified Dulbecco's medium with 20% fetal calf serum and 0.7% agar. 2×10^4 to 2×10^5 cells were added to 0.2 ml of the same culture medium and 0.4% agar and plated on to the base layer. Cytostatic drugs were applied by continuous exposure (drug overlay) in 0.2 ml medium. In each assay, six control plates received the vehicle only, drug-treated groups were plated in triplicate in three or six concentrations.

Drug effects were expressed as percentage survival obtained by comparing the mean number of colonies in the treated plates with the mean number of colonies of control plates (T/C%). Anti-tumour activity was considered to be significant if the compound reduced colony formation to 30% or less of the control value.

7 different human tumour xenografts were used. CXF 1103 (colon cancer), LXFL 529 (large cell lung cancer), LXFS 538 (small cell lung cancer), MAXF 401 (breast cancer), MEXF 514 (melanoma), OVXF 899 and OVXF 1023 (both ovarian cancer) were established in our laboratory and have been extensively described previously [22, 23].

In vivo *studies*

Tumour xenografts. Two human ovarian carcinoma xenografts, PXN/109T/C and the cisplatin-resistant subline PXN/109T/CC, grown subcutaneously (s.c.) in female BALB/c nude mice, a small cell lung cancer xenograft LXFS 538, and a large cell lung cancer xenograft LXFL 529 grown s.c. in female NMRI nude mice were used. PXN/109T/C (which was derived from continuous *in vitro* cell line CH1 by s.c. injection of 5×10^6 cells) is sensitive to cisplatin and PXN/109T/CC (which was similarly derived from cell line CH1cisR) is resistant to cisplatin. Growth characteristic details of CH1cisR have been described previously [18, 24].

LXFS 538 is moderately sensitive to cisplatin and LXFL 529 is a cisplatin-sensitive tumour. Tumour characterisation and

sensitivity to standard drugs have been described elsewhere [22, 23].

Assessment of anti-tumour activity

ADJ/PC6. This was performed as described previously [25]. Briefly, 20 days after s.c. implantation of 1-mm^3 tumour fragments, mice (syngeneic female Balb/c) bearing comparably sized tumours were randomised (five per dose level and 10 vehicle-treated controls) and drugs were administered intraperitoneally (i.p.) as a single dose in water. After 10 days, tumours were dissected out, and tumour weights in control and treated groups were compared. Anti-tumour activity was defined in terms of a therapeutic index (TI), the ratio of 50% lethal dose (LD_{50}) to ED_{90} (the dose leading to a 90% reduction in tumour mass).

Human ovarian cancer xenografts. Female BALB/c nude mice bearing comparably sized tumours (typically around 8 mm largest diameter) were randomised into groups of 6 (for treatment) or 10 (for controls). The drug was dissolved in water immediately before i.p. injection. Drugs were administered once daily at three dose levels on days 0, 7, 14 and 21 (q7dx4). Tumours were measured weekly until their starting volume had doubled [26, 27]. The tumour volumes were calculated by the formula $V = (a \times b^2)/2$ where a is the larger diameter and b the smaller. Tumour volumes were normalised with respect to their starting volumes and graphs of the relative tumour volumes against time were plotted.

Tumour inhibition was determined at day 28 by dividing tumour volumes of treated mice by the control values (T/C), and tumour growth delay (T-C) was the difference in days to double the initial tumour volume.

Human lung cancer xenografts. Female NMRI nude mice bearing comparably sized tumours (mean tumour diameter about 6–8 mm largest diameter) were randomised into groups of five (treated and controls). Cisplatin and cycloplatam were injected intravenously. Drugs were administered once daily on days 0, 4 and 8 (q4d×3). Tumours were measured weekly until day 28 or until their starting volume had doubled. The tumour volume was calculated by the formula $V = (a \times b^2)/2$. Tumour volumes were normalised with respect to their starting volumes and graphs of the relative tumour volumes against time were plotted [23, 28, 29]. Growth curves were analysed in terms of maximal tumour inhibition (treated/control, T/C) and growth delay (T-C).

Statistical analysis

The statistical data analysis of the *in vivo* investigations was performed by the *t*-test. All data of relative tumour volume (RTV) were logarithmically converted and a *t*-test applied, comparing each treatment group with controls.

RESULTS

In vitro *anti-tumour activity*

Table 1 shows the *in vitro* anti-tumour activity of cycloplatam in comparison to cisplatin against 15 cancer cell lines, including six pairs of parent and derived cisplatin-resistant cell lines, using the sulphorhodamine B assay. Overall, cycloplatam showed a cytotoxicity and cell line ranking similar to cisplatin. Mean IC_{50} values (μM) of cycloplatam were 5.7 (range 0.37–19) and of cisplatin 4.3 (range 0.18–24), respectively. For all six resistant cell lines, the resistance factor of cisplatin was higher than that of cycloplatam. The 41M/41McisR model, in particular, showed no cross-resistance to cycloplatam, and OVCAR-3/OVCAR-3cisR showed only partial cross resistance. In two resistant sublines, cycloplatam had higher cytotoxicity than cisplatin. For all resistant sublines, cycloplatam had lower resistance factors.

Further experiments (Table 2) confirmed the *in vitro* activity of cycloplatam in a clonogenic assay using human tumour xenografts. In six different tumour types (colon, lung, large cell and small cell, melanoma, breast, ovarian) *in vitro* activity was

Table 1. In vitro *cytotoxicity of cisplatin and cycloplatam against* 15 *cancer cell lines using the SRB assay*

Cell line	Tumour type	Cisplatin IC_{50} (μM)	Cisplatin Fold-resistance*	Cycloplatam IC_{50} (μM)	Cycloplatam Fold-resistance*
HX/62	Ovarian	13	—	13	—
SKOV-3	Ovarian	3.7	—	12.5	—
PXN/94	Ovarian	2.4	—	1.3	—
41M	Ovarian	0.3	—	1.7	—
41McisR	Ovarian	0.95	3.2	1.3	0.76
CH1	Ovarian	0.18	—	0.37	—
CH1cisR	Ovarian	0.6	3.3	0.9	2.4
OVCAR-3	Ovarian	5.7	—	11.8	—
OVCAR-3cisR	Ovarian	24	4.2	19	1.6
A2780	Ovarian	0.47	—	0.38	—
A2780 DDP	Ovarian	6.3	13.4	2.1	5.6
HX/155	Cervix	0.6	—	6.0	—
HX/155cisR	Cervix	4.6	7.7	13.2	2.2
GCT27	Testicular	0.27	—	0.37	—
GCT27cisR	Testicular	0.94	3.5	1.0	2.7
Mean		4.3	5.9	5.7	2.5

* Fold-resistance = IC_{50} cisR line/IC_{50} parent line.

Table 2. Colony inhibition by cycloplatam in vitro *against 7 human tumour xenografts*

Tumour	Test/control (%) at drug concentration (μg/ml) 0.01	0.1	1.0	10.0	100.0
CXF1103*			47	8	10
LXFL529	61	14	4	2	3
LXFS538			6	3	6
MAXF401			36	21	17
MEXF514			19	6	5
OVXF899			67	8	3
OVXF1023	29	20	20		
Active†/total (%)	1/2 (50)	2/2 (100)	4/7 (57)	6/6 (100)	6/6 (100)

* CXF, colorectal cancer xenograft; LXF, lung; L, large cell, S, small cell; MAXF, breast; MEXF, melanoma; OVXF, ovarian cancer xenograft.
† Active only if T/C < 30%.

Table 3. Colony inhibition by cisplatin in vitro *against 7 human tumour xenografts*

Tumour	Test/control (%) at drug concentration (μg/ml) 0.1	0.3	1.0
CXF1103*	69	70	54
LXFL529	28	15	3
LXFS538	37	17	10
MAXF401	66	57	21
MEXF514	42	44	14
OVXF899	91	104	45
OVXF1023	38	26	9
Active†/total (%)	1/7 (14)	3/7 (43)	5/7 (71)

* CXF, colorectal cancer xenograft; LXF, lung; L, large cell, S, small cell; MAXF, breast; MEXF, melanoma; OVXF, ovarian cancer xenograft. † Active only if T/C < 30%.

established, starting at a dose level of 0.01 μg/ml. At a dose level of 1 μg/ml activity, T/C < 30% was observed in 4 of 7 tumours. Cisplatin showed, at the same dose level, activity in 5 of 7 tumours (Table 3). Mean IC_{70} values (70% inhibition of colony formation compared to untreated controls) of cycloplatam were 0.54 μg/ml (1.25 μM) and of cisplatin 0.42 μg/ml (1.4 μM), respectively.

In vivo *anti-tumour activity*

ADJ/PC6. Table 4 summarises anti-tumour activity and LD_{50} values in the murine ADJ/PC6 plasmacytoma model following i.p. single dose administration. No random animal deaths occurred in the test. Cycloplatam had 6-fold higher LD_{50} values than cisplatin, however, the dose required to reduce the tumour mass by 90% (ED_{90}) was 14-fold higher. This resulted in an approximately 2-fold smaller therapeutic index in comparison to cisplatin's.

Table 4. In vivo *anti-tumour activity of cisplatin and cycloplatam against ADJ/PC6 plasmacytoma*

Drug	Single i.p. dose (mg/kg) LD_{50}*	ED_{90}	TI
Cycloplatam	62†	8.4	7.3
Cisplatin	11	0.6	18

* LD_{50}, 50% of the lethal dose; ED_{90}, dose required to reduce the tumour mass by 90%; TI, therapeutic index LD_{50}/ED_{90}. † Data were obtained from two independent experiments.

Ovarian cancer xenografts. The *in vivo* anti-tumour activity of cycloplatam against two ovarian carcinoma xenografts PXN/109T/C and PXN/109T/CC is shown in Table 5. PXN/109T/C is a cisplatin sensitive xenograft, exhibiting a growth delay of approximately 40 days following i.p. administration of 6 mg/kg cisplatin (maximal tolerated dose) given weekly for 4 weeks, and PXN/109T/CC is a cisplatin-resistant model, exhibiting only 4 days growth delay. Cycloplatam was well tolerated up to 20 mg/kg (q7d×4, i.p.). There were no drug-related deaths, but at the top dose a loss of body weight was observed. Cycloplatam was less active than cisplatin, producing, at 20 mg/kg (maximal tolerated dose), a growth delay of 19 days in PXN/109T/C and 6 days in PXN/109T/CC.

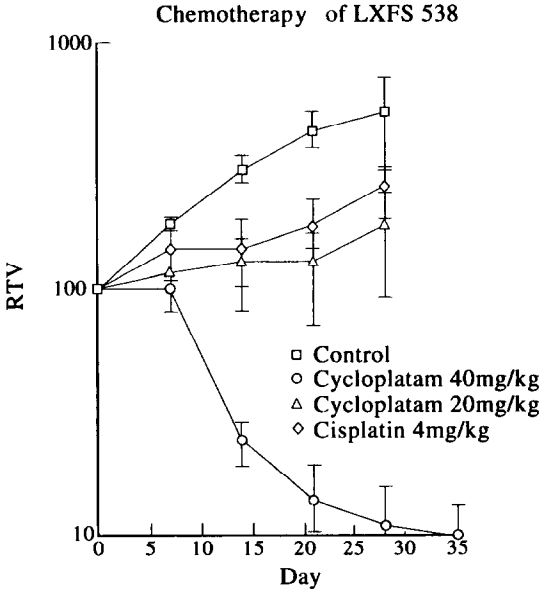

Figure 2. Growth curves of LXFS 538 in nude mice treated with cycloplatam or cisplatin once every 4 days (day 0, 4 and 8), i.v. (5 animals with 8–10 tumours/group). RTV, relative tumour volume.

Table 5. Activity of cycloplatam against two ovarian carcinoma xenografts in vivo

Tumour	Drug	Dose level (mg/kg)*	Tumour inhibition T/C %†	Growth delay (days)
PXN/109T/C	Cycloplatam	20	35‡	19
		10	82§	3.8
		5	98§	1.5
PXN/109T/CC	Cycloplatam	20	84§	6.3
		10	110§	1.40
		5	149§	<1

* Schedule days 0, 7, 14 and 21 intraperitoneally (6 animals with 10–12 tumours/group). † On day 28. ‡ Significant (t-test, $P < 0.01$) compared to control group. § Not significant (t-test, $P < 0.01$) compared to control group.

Lung cancer xenografts. Table 6 shows anti-tumour activity of cycloplatam and cisplatin against two lung cancer xenografts. LXFS 538 is moderately sensitive to cisplatin and LXFL 529 is a cisplatin-sensitive model, exhibiting strong tumour volume inhibition administered at 4 mg/kg,q4d×3, i.v. (maximal tolerated dose). Cycloplatam was administered at two dose levels (20 and 40 mg/kg, q4d×3, i.v.) which were well tolerated. Cycloplatam was inactive for LXFL 529 up to the maximal tolerated dose (40 mg/kg, q4d×3, i.v.). In LXFS 538 (Figure 2), cycloplatam produced a partial remission at the maximal tolerated dose. These results demonstrate a different spectrum of anti-tumour activity for cycloplatam and cisplatin in lung cancer.

DISCUSSION

A novel platinum compound, cycloplatam, has been evaluated for anti-tumour activity *in vitro* and *in vivo* in comparison with cisplatin. In 15 cell lines of mainly ovarian origin, both compounds exhibited similar cytotoxicity. The mean IC_{50} values were 5.7 μM for cycloplatam and 4.3 μM for cisplatin. However, carboplatin, structurally more related to cycloplatam, showed a 5–10 times lower activity (data not shown) in most of these cell lines related to a higher stability and lower reactivity. Furthermore, cycloplatam exists in equilibrium between two isomeric forms (Figure 1) with a shifting hydroxyl group, which could effect a higher reactivity of cycloplatam. However, the importance of both isomeric forms, caused by the shifting hydroxyl group, is still unknown.

In six pairs of parental and cisplatin-resistant cell lines, we observed a lower resistance factor for cycloplatam than for cisplatin. The 41M/41McisR model showed no cross-resistance to cycloplatam and OVCAR-3/OVCAR-3cisR only partial cross-resistance. In the other ovarian cell lines, only small differences in the anti-tumour activities of cycloplatam and cisplatin were seen.

Further *in vitro* studies in 7 human tumour xenografts, studied using the clonogenic assay, confirmed the activity of cycloplatam. Mean IC_{70} values of cycloplatam and cisplatin were similar, which demonstrates that both compounds have a similar potency *in vitro*.

Summarising the *in vitro* results, cycloplatam has a similar spectrum of anti-tumour activity as cisplatin. However, the studies in cisplatin-resistant cell lines showed a clear advantage for cycloplatam. The main goal in developing cisplatin compounds is to circumvent cisplatin resistance. From this point of view, cycloplatam is a candidate for further investigations.

Since *in vitro* activity was established, we investigated cycloplatam in various tumour models *in vivo*. In the ADJ/PC6 murine model, an approximately 2-fold smaller therapeutic index was observed for cycloplatam compared to cisplatin. Anti-tumour activity *in vivo* against two human ovarian carcinoma showed no clear advantage for cycloplatam. In the PXN/109T/C model, cisplatin, at the maximal tolerated dose, produced a growth delay of 40 days [24], while cycloplatam produced a growth delay of 19 days. In the cisplatin-resistant model PXN/109T/CC, cisplatin showed a growth delay of 4 days [24] and cycloplatam showed a slightly higher growth delay of 6.3 days. In this pair of parent and resistant ovarian cancer xenografts, cycloplatam did not circumvent cisplatin resistance.

In lung cancer xenografts, we observed a completely different spectrum of activity between cycloplatam and cisplatin *in vivo*. LXFS 538 is moderately sensitive to cisplatin. The maximal

Table 6. Activity of cycloplatam and cisplatin against lung cancer xenografts in vivo

Tumour	Drug	Dose level (mg/kg)*	Tumour inhibition T/C %†	Growth delay (days)
LXFS 538	Cycloplatam	40	2†‡	>28§
		20	29†‡	20
	Cisplatin	4	41†‡	14
LXFL 529	Cycloplatam	40	72‖¶	<1
		20	82‖¶	<1
	Cisplatin	4	23‡‖	18

* Schedule days 0, 4, and 8 intravenous (5 animals with 8–10 tumours/group). † On day 28. ‡ Significant (t-test, $P < 0.01$) compared to control group. § Partial remission still on day 28. ‖ On day 14 (animals were sacrificed, excessive tumour growth except cisplatin group). ¶ Not significant (t-test, $P < 0.01$) compared to control group.

tolerated dose of cisplatin (4 mg/kg, q4d×3, i.v.) only produced tumour stasis up to 5 days after the last therapy. Cycloplatam could be administered in these tumour models up to 40 mg/kg, using a different mouse strain (NMRI nude mice) than in ovarian cancer xenografts (Balb/c nude mice) tolerant of higher doses of cycloplatam. The maximal tolerated dose of 40 mg/kg (q4d×3, i.v.) produced a partial remission of the tumour, which persisted 30 days after last therapy. LXFL 529, a cisplatin-sensitive model, appeared to be resistant to cycloplatam. The top dose of 40 mg/kg (q4d×3, i.v.) produced only a smaller tumour growth inhibition. However, cisplatin at a dose of 4 mg/kg (q4d×3, i.v.) showed a strong tumour volume inhibition. In these lung cancer xenografts, there was no cross activity between cycloplatam and cisplatin. The very promising results in the lung cancer xenograft LXFS 538 led us to select cycloplatam for further preclinical investigations, in order to establish the anti-tumour activity in, for example, gastric tumour models *in vivo* in comparison with cisplatin. If a different spectrum of anti-tumour activity can also be established in other tumour types, then cycloplatam would be a candidate for clinical phase I investigation. Independently of our results, cycloplatam is being studied in clinical phase II trials in Russia.

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