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Titanocendichloride Activity in Cisplatin and Doxorubicin-resistant Human Ovarian Carcinoma Cell Lines

A. Harstrick, H.-J. Schmoll, G. Sass, H. Poliwoda and Y. Rustum

The activity of a new organometallic compound, titanocendichloride, was evaluated in doxorubicin- and cisplatin-resistant human ovarian carcinoma cell lines *in vitro*. Titanocendichloride showed no cross resistance to doxorubicin in two multidrug resistant sublines of A2780. Furthermore, the cell line A2780 CP3, which is about 20-fold resistant to cisplatin was only 2.5-fold resistant to titanocendichloride, indicating a lack of cross resistance between the two metal compounds. These results were confirmed *in vivo* where titanocendichloride showed a much stronger inhibitory effect in cisplatin-resistant human ovarian carcinoma xenografts than cisplatin.
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INTRODUCTION

A VARIETY OF agents have demonstrated antitumour activity against ovarian carcinoma, including cisplatin, carboplatin, cyclophosphamide, melphalan and doxorubicin [1–3]. Currently, no agents are available with documented activity in cisplatin- and doxorubicin-resistant ovarian carcinoma.

A variety of organometallic early transition metal compounds possess antitumour activity in experimental models [4]. These agents have a transition metal as their central atom, bound to two planar cyclopentadienyl rings and to two *cis*-bound ligands, mostly halides (Fig. 1). From this class, titanocendichloride was the most active agent with high activity against Ehrlich ascites tumour, colon 38 adenocarcinoma, B16 melanoma, Lewis lung carcinoma and human breast, non-small cell lung and colon carcinoma xenografts [5–8].

Based on these results and the apparent lack of nephro- and neurotoxicity [4, 5], the agent was further developed and is currently in clinical phase I trials in Germany.

MATERIALS AND METHODS

Drugs and chemicals

Doxorubicin was from Adria Laboratories (Columbus, Ohio, U.S.A.); cisplatin and D-L-buthionine sulphoximin (BSO) were from Sigma (St. Louis, Missouri, U.S.A.). Titanocendichloride was provided by Medac (Hamburg, Germany). All drug solutions were prepared fresh immediately before use.

Cell lines

The human ovarian carcinoma cell line A2780 (A2780-WT for "wild type") established from a non-pretreated patient with ovarian carcinoma and the cisplatin-resistant variants, designated A2780-CP2 and A2780-CP3, were obtained from R. Ozols and T. Hamilton (Fox Chase Cancer Center, Philadelphia, U.S.A.) [9]. A2780-CP2 and -CP3 cells are about 10-fold and 20-fold cisplatin-resistant, respectively [10, 11]. The cell lines A2780-Dx1 and A2780-Dx5, which are 5-fold and 78-fold doxorubicin-resistant, respectively, were established by M.A. Jamali in our laboratory by repeated exposure of A2780-WT cells to increasing concentrations of doxorubicin [12]. Both cell lines express the multiple drug resistance phenotype.

Cytotoxicity assay

For assessment of cytotoxicity, the sulphrhodamin B assay was used [13]. Cells were seeded at a density of 1000 cells/well in 96-well microtitre plates (Falcon) and allowed to attach overnight. Then 100 μl of medium containing appropriate drug concentrations were added and the cells were incubated for 96 h. After washing twice with phosphate buffered saline (PBS) the cells were fixed with 100 μl 10% acetic acid for at least 1 h and the staining procedure was performed as originally described. Eight wells were used for one concentration of drug and all experiments were performed in duplicate. The drug concentration which inhibited cell growth by 50% (IC_{50}) was determined from semi-logarithmic dose–response plots.

Glutathione depletion was achieved by adding BSO in a final concentration of 30 μmol/l which lead to a 70–90% reduction of cellular glutathione and is not cytotoxic itself.

Evaluation of cytotoxic activity in vivo

Cells from line A2780-CP_2 were injected subcutaneously in the right flank of 6–8-week-old, female NMRI nude mice (1×10^7 viable cells/mouse). After 3 weeks the mice were divided into treatment groups of 8–10 animals. Treatment consisted of either cisplatin 4 mg/kg/day intraperitoneally (i.p.) on days 1, 3 and 5 or titanocendichloride (dissolved in maleate

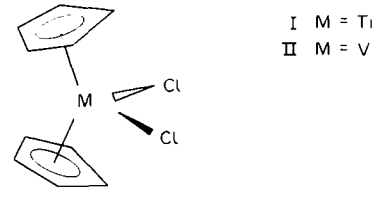

Fig. 1. Structure of organometallic compound; I = titanocendichloride.

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Table 1. IC_{50} *values for cisplatin, doxorubicin and titanocendichloride in cell lines A2780 WT, A2780 Dx1, A2780 Dx5, A2780 CP2 and A2780 CP3*

	IC_{50}(μmol/l)		
	Cisplatin	Titanocendichloride	Doxorubicin
A2780-WT	0.20	34.0	0.0009
+BSO	0.13	13.0	0.0006
A2780-Dx1	0.18	25.0	0.0046
A2780-Dx5	0.18	40.0	0.0680
A2780-CP2	1.90	36.0	n.d.
A2780-CP3	4.20	90.0	0.01
+BSO	2.75	36.0	0.005

buffer) 40 mg/kg/day i.p. on days 1, 3 and 5. These two schedules are equitoxic (app. LD10) in NMRI-nude mice as determined in separate experiments using non-tumour bearing nude mice (data not shown).

RESULTS

Neither of the two metal compounds exhibited cross-resistance to doxorubicin in the multidrug resistant cell lines A2780-Dx1 and A2780-Dx5 (Table 1). Furthermore, titanocendichloride did not appear cross-resistant to cisplatin in the 20-fold resistant line A2780-CP3.

Glutathione depletion increased the cytotoxicity of all three compounds in A2780-WT and A2780-CP3 cells. This effect was most pronounced for titanocendichloride where BSO almost completely reversed the 2.6-fold resistance in A2780 CP3.

The *in vivo* activity of both metal compounds was evaluated in the cisplatin-resistant line A2780-CP_2, transplanted in NMRI nude mice. When given at an equitoxic dose, titanocendichloride resulted in a much stronger growth inhibition than cisplatin (Fig. 2). These data indicate that the two drugs also seem to be non-cross-resistant *in vivo*.

DISCUSSION

Early transition metal organometallic complexes represent a new class of chemicals that might possess meaningful antitumour activity. Structure-activity studies have shown that the central atom, e.g. titanium, can be replaced by other early transition metals (e.g. vanadium or hafnium) without significant loss of antitumour activity. The same applies for the two acido groups which can either be halides (F, Cl, Br); pseudohalides or certain carboxylato-groups like hydrogen-maleinate or trichloroacetate [15]. On the other hand, any modification of one or both cyclopentadienyl rings results in a significant loss of antitumour activity, indicating a crucial role of these ligands in the still unknown mechanism of action.

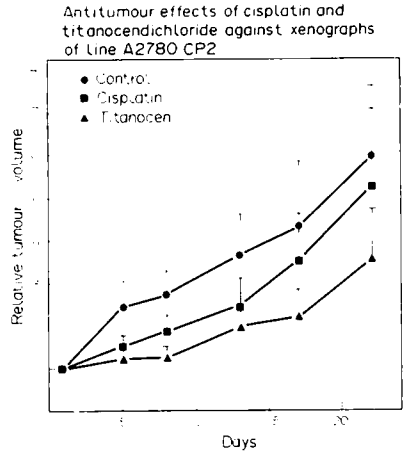

Fig. 2. Growth of A2780-CP2 xenografts treated with cisplatin or titanocendichloride.

So far the best characterised metallocene compound is titanocendichloride. Little is known, however, about its activity in comparison to other standard agents and about the cross-resistance pattern.

Both metal compounds, cisplatin and titanocendichloride, showed no cross-resistance to doxorubicin in the two p-glycoprotein expressing doxorubicin-resistant cell lines A2780-Dx1 and A2780-Dx5. Of even more importance is the apparent lack of cross resistance between titanocendichloride and cisplatin, as demonstrated in the two cisplatin-resistant ovarian carcinoma cell lines. Elevated cellular glutathione levels have been shown to contribute to the resistance to doxorubicin and cisplatin in A2780-CP3 cells [10, 11]. Glutathione depletion led to a significant increase in the cytotoxicity of titanocendichloride in the sensitive line A2780-WT, as well as in the resistant line A2780-CP3. This indicates that the glutathione pathway could play an important role in the metabolism and detoxification of organometallic complexes. Given the high activity of titanocendichloride against a variety of experimental tumours *in vivo* and its lack of cross resistance to doxorubicin and cisplatin as demonstrated in these studies *in vitro* and *in vivo*, this compound could be an interesting new agent for a variety of human tumours, including drug-resistant ovarian carcinomas.

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Long Term Results of Treatment in Patients with Extragonadal Germ Cell Tumours

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From 1979 to 1991 56 patients with extragonadal germ cell tumours (EGCT) received cisplatin based chemotherapy. From 16 patients with seminomatous EGCT 13 achieved complete remission (CR) with chemotherapy alone, 2 with additional radiotherapy with final CR rate of 94%. 5 (31%) patients developed relapses and at a median follow-up of 38 (5–103) months 11 (69%) are alive and 10 (62%) have no evidence of disease (NED). Only 7 patients with non-seminomatous EGCT reached CR with chemotherapy alone and 8 more with additional chemotherapy or surgery. Overall CR was 37% and 3 (20%) relapses have been observed. At a median follow-up of 26 (3–114) months 14 (35%) are alive and remain free of disease, 26 (65%) have died. By univariate analysis seminomatous EGCT patients had a significantly greater likelihood of achieving a CR, for non-seminomatous EGCT *BEP* induction chemotherapy was superior to VAB-6, and NSEGCT patients with serum levels > 2000 ng/ml had worse prognosis. Current staging systems are insufficient to predict the treatment outcome in EGCT.
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INTRODUCTION

EXTRAGONADAL GERM cell tumours (EGCT) are uncommon neoplasms. They share serological and morphological characteristics with the primary germ cell tumours of the testis and consequently their treatment is similar. Considerable success has been achieved since introduction of cisplatin based chemotherapy in the treatment of testicular cancer, but results are inferior for EGCT [1–9]. This report describes the clinical experience of the Cancer Research Center (CRC) of the Russian Academy of Medical Sciences in patients with ECGT.

MATERIALS AND METHODS

From 1979 to 1991, 56 patients with EGCT and a mean age of 29 (16–60) years, were treated and evaluated for response and survival. We defined EGCT as a germ cell tumour (GCT) arising in the mediastinum, retroperitoneum and other sites, without demonstrable gonadal tumour at presentation as determined by testicular ultrasound and/or negative testicular biopsy specimens. Patients with undifferentiated tumour or with histological diagnosis of the mediastinal, or retroperitoneal tumour with elevated serum markers, have been included in the analysis. Before treatment all patients had a complete history and physical examination, blood chemistry and complete blood count, chest X-ray and computed tomography (CT) scans of the lungs and abdomen, as well as an abdominal ultrasound. Radioimmunoassay techniques were used for quantitative determination of alpha fetoprotein (AFP) and human chorionic gonadotrophin (HCG). Serum values of less than 13 ng/ml and 10 ng/ml, respectively, were considered normal. Estimations of extent of the disease were based on the Indiana staging system [10] and the probability of treatment outcomes was calculated as previously described [11].

Characteristics of the patients are summarised in Table 1. The mean and range AFP levels (ng/ml) for each subgroup were: 9 patients with different subtypes of non-seminomatous EGCT (NSEGCT): 6880 (18–16000); 6 patients with GCT: 1014 (14–4000); 5 patients with undifferentiated tumours: 1388 (14–1600); and for 5 patients with unknown histology: 1826 (14–8000). In all patients the tumour was localised in either the mediastinum, or retroperitoneum. However, 2 patients had EGCT of axillary or cervical lymph nodes and pelvic lymph node involvement was also observed in 1 case.

26 patients had been previously treated with chemotherapy, debulking surgery and radiotherapy or a combination thereof. None had been previously treated with cisplatin-based combination chemotherapy and were referred after failing initial treatment. The patients were treated with various cisplatin-based regimens previously described for the treatment of primary testicular cancer, VAB-6 [12], PVB+ doxorubicin [13], BEP [14], CP [15], and with other combinations (cisplatin + ifosfamide, cisplatin + vinblastine + cyclophosphamide).

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