Activity of [1,2-di(cyclopentadienyl)-1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride against tumor colony-forming units

Olaf Oberschmidt[a], Axel-R. Hanauske[a], Franz-Josef K. Rehmann[b], Katja Strohfeldt[b], Nigel Sweeney[b] and Matthias Tacke[b]

[1,2-di(cyclopentadienyl)-1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride is a newly synthesized transition metal-based anti-cancer drug. We studied the anti-tumor activity of this drug (final concentrations: 25, 250 and 2500 μmol/l) against freshly explanted human tumors, using an *in vitro* soft agar cloning system. A total of eight tumor samples were evaluated using 1-h exposures. Additionally, the breast carcinoma cell line MCF-7 was examined with regard to sensitivity. The tested compound was markedly active against one renal cancer sample, whereas other renal tumors were resistant. Concentration-dependent anti-tumor activity was demonstrated for all samples except for melanoma. At concentrations of 250 μmol/l or less, the compound was less active than cisplatin or equally active at 0.2 μg/ml, whereas at 2500 μmol/l it showed a significant cytotoxic activity against a wide spectrum of tumor types. The highest activity was observed against renal carcinomas (three of three tumor specimens inhibited at 2500 μmol/l). Sensitivity was also highly remarkable in the breast cancer cell line MCF-7 inhibited in a range of 25–2500 μmol/l, whereas melanoma cells seemed to be profoundly resistant. Further clinical development of this drug appears warranted because of the broad cytotoxic activity shown. *Anti-Cancer Drugs* 16:1071–1073 © 2005 Lippincott Williams & Wilkins.

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[a]Allgemeines Krankenhaus St Georg, Hamburg, Germany and [b]Conway Institute of Biomolecular and Biomedical Research, Chemistry Department, Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland.

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Correspondence to O. Oberschmidt, Allgemeines Krankenhaus St Georg, Lohmühlenstraße 5, 20099 Hamburg, Germany.
Tel: +49 40 181885 4094; fax: +49 40 181885 2388;
e-mail: tian64@gmx.de

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Introduction

Despite the success of cisplatin and related platin anti-tumor agents, the movement of other transition metal anti-cancer drugs towards the clinic has been exceptionally slow [1–3]. Metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo show remarkable anti-tumor activity [4,5]. However, the efficacy of Cp_2TiCl_2 in phase II clinical trials in patients with metastatic renal cell carcinoma [6] or metastatic breast cancer [7] was too low to be pursued. Very recently, more synthetic effort has been employed to increase the cytotoxicity of titanocene dichloride derivatives [8–12]. A novel method starting from titanium dichloride and fulvenes [13–16] allows direct access to highly substituted *ansa*-titanocenes [17–20]. Using this method, we have synthesized [1,2-di(cyclopentadienyl)-[1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride (**1**), which has an IC_{50} value of 2.7×10^{-4} M when tested for cytotoxic effects on the LLC-PK cell line [21]. The structure of **1** is shown in Fig. 1. Heteroaryl [22] and methoxyphenyl [23,24] substituted *ansa*-titanocenes show similar IC_{50} values. The purpose of our present study was to evaluate the extent of cytotoxicity and anti-tumor activity of **1**. Using a capillary soft agar cloning system, we studied the anti-tumor activity of **1** on freshly explanted tumor cells and the established breast carcinoma cell line MCF-7, and compared the activity of **1** with cisplatin.

Materials and methods

Anti-tumor agents

Compound **1** was synthesized as described previously [21]. Solutions were prepared freshly in double-enriched CMRL medium including 9% DMSO. CMRL (2 ×) contains 500 ml CMRL 1066 medium (Gibco, Grand Island, New York, USA), 75 ml inactivated horse serum (Gibco), 10 ml inactivated FBS (Gibco), 10 ml 2 mmol/l L-glutamine (Gibco), 6 ml 1 mmol/l non-essential amino acids (Gibco), 6 ml hydrocortisole (400 ng/ml; Sigma, St Louis, Missouri, USA), 5 ml 100 mmol/l sodium pyruvate (Gibco), 5 ml vitamin C (30 mmol/l) (Merck, Germany), 5 ml penicillin/streptomycin solution (Gibco), 27 μl catalase solution (10^6 U/ml) (Serva, Germany), 82 μl epidermal growth factor solution (100 ng/ml) (Gibco), 5 ml 1 M HEPES solution (Gibco), 5 ml 100 mmol/l sodium pyruvate

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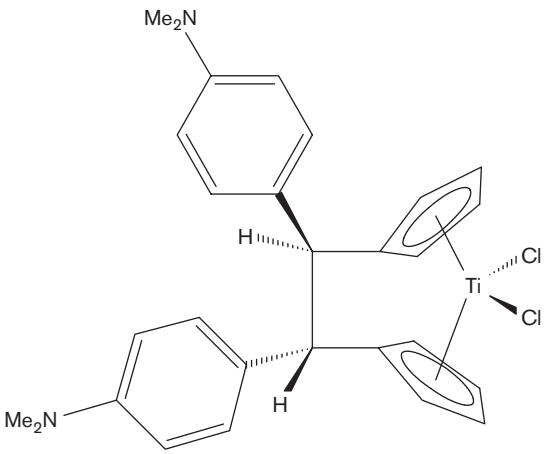
(Gibco), 5 ml 2 mmol/l (Gibco) and 7.5 ml asparagine solution (6.6 mg/ml) (Merck).

Compound **1** was studied at final concentrations of 25, 250 and 2500 μmol/l. Cisplatin was prepared as in clinical applications and was used at final a concentration corresponding to 0.1 times the clinically observed peak plasma concentration: 0.2 μg/ml. Stock solution of cisplatin was stored at −80°C prior to use.

Capillary soft agar cloning system

For the human tumor cloning assay (HTCA), cells were obtained from freshly biopsied tumor specimens or from pleural or ascites effusions that were achieved by sterile standard procedures as part of routine clinical measures. The generation of single-cell suspensions followed instructions as described previously [25]. HTCA experiments were performed as described elsewhere [26,27]. Tumor cells were exposed to **1** for 1 h in all investigations. In negative controls, the anti-tumoral compound was substituted by 0.9% NaCl. Positive controls contained 10^{-3} mol/l ammonium vanadate to inhibit cell growth. Colony formation was evaluated with an inverted microscope after an incubation period of 21 to 28 days at 37°C, 5% CO_2 and 100% humidity. Experiments were considered to be evaluable if positive controls demonstrated 30% or less colony formation compared to NaCl control.

Fig. 1



Structure of titanocene **1**.

Statistical analysis

Data were calculated as means and SDs of six replicates for each concentration and all controls. Percentage colony survival was calculated by determining cells exposed to anti-tumor agent relative to calculated cells in the untreated negative control. Inhibition was defined as significant if colony formation was 0.5 or less times the negative control.

Results and discussion

The anti-tumor effect of **1** was studied in a total of eight tumors and one established breast cancer cell line following a 1-h short-term exposure. The total and evaluable experiments are listed in Table 1, while the most promising results are summarized in Fig. 2. At 25 μmol/l **1**, 32% of the breast carcinoma (MCF-7) cells died, while the medium concentration of 250 μmol/l induced 59% cell death. Following the highest concentration of 2500 μmol/l, 90% of the MCF-7 cells were killed. In the case of pancreatic cancer, colony survival decreased in a concentration-dependent manner (46% at 250 μmol/l, 13% at 2500 μmol/l). Ovarian cancer demonstrated marked sensitivity to **1** with 55% colony survival at 25 μmol/l and 12% survival at 250 μmol/l compared to control. Similar observations were made in other cancers of the urogenital tract. In renal cancer, the low concentration killed 74% of tumor colony-forming units, and the medium and high concentrations were associated with 86 and 88% decrease in colony formation. This is particularly encouraging since renal cancer does not respond to cisplatin treatment. For cervical cancer, 27% of the colony-forming units died at the low concentration, and 100% of the cells were killed at the medium and high concentrations.

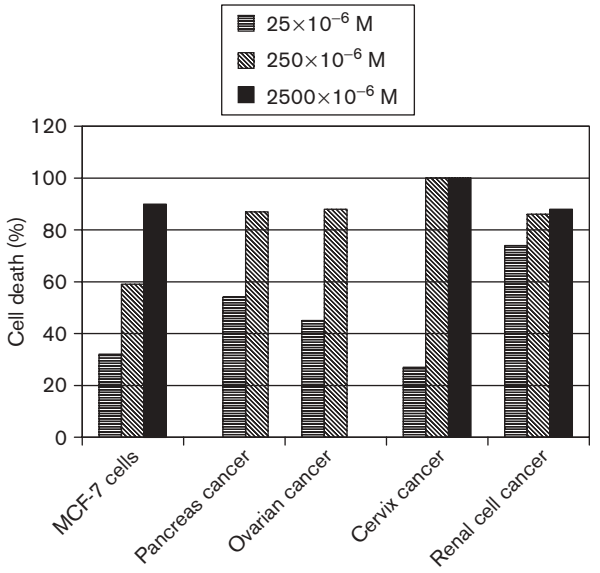
The inhibitory effect of **1** was also directly compared to cisplatin (0.1 times the clinically achievable peak concentration, 0.2 μg/ml). In most samples, titanocene **1** (2500 μmol/l) showed superior inhibition compared to cisplatin: cell death induced by titanocene **1** versus cell death induced by cisplatin was 100 versus 47% for cervical cancer, 90 versus 53% for MCF-7 and 88 versus 1% for

Table 1 Inhibitory effect of 1 on short-term *in vitro* growth of primary tumor cells and the breast cancer cell line MCF-7

Tumor type	No. of specimens inhibited[a]/no. of specimens evaluable			
	Titanocene **1** (25 μmol/l)	Titanocene **1** (250 μmol/l)	Titanocene **1** (2500 μmol/l)	Cisplatin (0.2 μg/ml)
MCF-7	0/1	1/1	1/1	1/1
Melanoma	0/1	0/1	0/1	0/1
Pancreatic cancer	0/1	1/2	2/2	1/1
Cervix	0/1	1/1	1/1	0/1
Ovarian cancer	0/1	1/1	–	–
Kidney	1/3	1/3	3/3	1/2
Total	1/8 (13%)	5/9 (56%)	7/8 (88%)	3/6 (50%)

[a]Survival of tumor colony-forming units 50%.

Fig. 2



Cytotoxicity data of **1** in MCF-7 and HTCA cell tests.

renal cancer samples. Neither agent (cisplatin and **1**) affected melanoma cells.

From these results it is concluded that breast and pancreatic cancers are likely to also be clinically sensitive to **1.** Our results encourage further clinical and preclinical investigations to determine the anti-tumor spectrum of **1.** In particular, cancers of the urogenital tract appear to be sensitive and should be further studied using xenograft models.

Outlook

Because of the also promising results with renal cell and breast cancer, our best titanocene will be tested by Dr Iduna Fichtner from the MDC in Berlin in a corresponding mouse model (CAKI-1) and by Professor Volker Schirrmacher from the DKFZ in Heidelberg in a MCF-7 mouse model in the near future.

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