Human tumour xenografts in nude mice: chemotherapy trials with titanocene dichloride in different dosages

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Summary

Purpose: In this study new cytostatic therapies with titanocene dichloride in different dosages for the treatment of ovarian cancer are analyzed on human tumour xenografts in nude mice. The aim was to compare the effects of different dosages of titanocene dichloride on the growth of human tumour xenografts and nude mice body weight.

Methods: Biopsy material from one human ovarian carcinoma was expanded and transplanted into 52 nude mice. The treatment protocol included one experiment that consisted of the following six treatment groups: titanocene dichloride 3 x 10 mg/kg, titanocene dichloride 3 x 20 mg/kg, titanocene dichloride 3 x 30 mg/kg, titanocene dichloride 1 x 30 mg/kg, titanocene dichloride 1 x 40 mg/kg and a control group treated with 0.9% saline. Treatment groups were evaluated in terms of average daily increase in tumour volume and average daily body weight increase of nude mice. The slope factors α and β of the body weight and tumour volume changes were calculated.

Results: Titanocene dichloride in the dosage of 3 x 30 mg/kg and 3 x 20 mg/kg brought about a significant reduction in tumour volume ($p<0.05$) compared to the control group and to the treatment group under medication with titanocene dichloride 1 x 30 mg/kg. There were no significant changes in the body weight of nude mice.

Conclusion: We found titanocene dichloride to be effective in the reduction of tumour volume increase in nude mice. Titanocene dichloride could be an active chemotherapeutic drug in women with ovarian carcinoma not responding to standard therapies.

Key words: Nude mice; Titanocene dichloride; Tumour volume; Body weight.

Introduction

Today, chemotherapy is regarded as one of the most effective and most important treatment measures in ovarian carcinoma. In a large number of patients with ovarian carcinoma, survival depends on the efficacy of cytostatic therapy and very much less on all other measures [1]. A variety of agents have demonstrated antitumour activity against ovarian carcinoma, including cisplatin and its analogue carboplatin, paclitaxel, cyclophosphamide, treosulfane, ifosfamide and gemcitabine [2]. An additional characteristic of platinum derivatives, paclitaxel, gemcitabine and etoposide, is that they can still be effective in resistance to alkylating agents and in a recurrence after cisplatin pre-treatment. In the primary treatment, cisplatin combinations are used preferentially owing to the more favourable results with regard to rate of remission, median duration of remission and median survival time [3]. In about 50% of patients with an ovarian carcinoma, a major tumour reduction cannot be effected. Their prognosis is especially unfavourable. It is therefore logical to look for more effective chemotherapies in these cases.

Titanocene dichloride is an early transition metal complex containing the intact bis(cyclopentadienyl)-titanium unit. The compound showed significant antitumour activity in a broad range of tumour models tested *in vivo* and *in vitro* [4-10]. Results of animal experiments confirm a primary interaction of titanium-containing metabolites derived from titanocene complexes with nucleic acid molecules, especially with DNA. They suggest the formation of aggregates between nucleic acids and titanium containing metabolites which are obviously eliminated from the nuclei and incorporated into cytoplasmic lysosomes [11]. Bone marrow is usually not affected by the antiproliferative activity of titanocene [12]. Interestingly it was shown, that titanocene dichloride is active against cisplatin-resistent tumour cells *in vitro* and *in vivo* [7]. Clinical phase I trials have been conducted showing that nephrotoxicity and hepatotoxicity are of dose-limiting character dependent on the dosing regimen application [13, 14]. Titanocene dichloride is currently under clinical phase II evaluation in different human tumour histologies.

The present study was performed to investigate the effect of different dosages of titanocene dichloride on tumour volume and body weight of nude mice in order to be able to appraise the efficacy and aggressiveness of titanocene dichloride in different dosages and its side-effects.

Materials and Methods

Chemotherapeutics: Titanocene dichloride was provided by Medac (Hamburg, Germany). All drugs were prepared fresh immediately before use.

Animals: Athymic nude mice at least 6 weeks old and derived from an independent company (Harlan-Winkelmann GmbH, Borchen, Germany) were used in all experiments. Mice were maintained under barrier conditions and given sterilised food (Altromin GmbH, Lage, Germany) and water.

Heterotransplantation of tumour into nude mice: Human tumour was obtained from only one patient as a fresh surgical spe-

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cimen. The tumour was cut into small fragments of about 20 mm^3 and implanted s.c. into both sides of nude mice. Usually, no major difference was observed between the growth on one side and growth on the contralateral side.

Drug Treatments: All treatments were administered intraperitoneally. Titanocene dichloride was given at doses of 3 x 10 mg/kg, 3 x 20 mg/kg, 3 x 30 mg/kg, 1 x 40 mg/kg and 1 x 30 mg/kg (see Table 1).

Characteristics of the primary tumour: Staging of ovarian cancer was carried out in accordance with the most recent FIGO-classification. The primary tumour stage was FIGO IIIc. The histology showed a dedifferentiated serous ovarian adenocarcinoma with the tumour stage pT3c pN0 GIII. Abdominal hysterectomy with bilateral adnexectomy, omentectomy, removal of pelvic lymph nodes and exploratory peritoneal excisions was performed. Postoperatively the patient received adjuvant chemotherapy with cisplatinum (100 mg/m^2) and treosulfan (5 g/m^2).

Procedures and design of the study: In one experiment tissue from one human ovarian cancer was transplanted into a total number of 52 mice. If transplantation proved successful, a treatment protocol was established which included mice receiving placebo (0.9% saline) and titanocene dichloride in the above mentioned doses. Sample sizes within the different treatment groups ranged from 8 to 10 animals. The protocol of the study was plannned as described in Table 1. At an interval of two days, the tumour volume and weight of the experimental animals were measured. The observation period was 7 days. In several groups of the experiment, some mice died before the end of observation. All animals were sacrificed at the end of the experiment. Tumours were measured in three perpendicular dimensions and their volumes were estimated using the formula (pi/6 x length x width x height). Treatment was administered when the median tumour volume reached about 600 mm^3.

Statistical Methods: All computations are based on average values from two implant sites. Δweight ($\Delta W\ t_x\text{-}t_0$) and Δvolume ($\Delta V\ t_x\text{-}t_0$) were determined by the difference of body weights and tumour volumes at the end (Wt_x/Vt_x) and at the beginning (Wt_0/Vt_0) of the experiment. Treatment groups were evaluated in terms of average daily increase in tumour volume and average daily body weight increase of nude mice based on slopes of least square regressions performed on individual animals. The slope factors α and β of the body weight (α) and tumour volume (β) changes within each group in the course of an experiment were calculated. This procedure was preferred to final values because observation durations differeed between groups and individuals (linear regression). Statistical comparisons were based on analysis of variance (ANOVA). Simple ANOVA was performed when comparing treatments within the experiment.

Table 1. — *Design of the study*

Experiment	Group	n	Chemotherapy	Dose (mg/kg)	Day of application	Period of observation (days)
1	1	8	titanocene	3 x 10 mg	1, 3, 5	7
	2	8	titanocene	3 x 20 mg	1, 3, 5	
	3	8	titanocene	3 x 30 mg	1, 3, 5	
	4	9	titanocene	1 x 30 mg	1	
	5	9	titanocene	1 x 40 mg	1	
	6	10	0.9% saline		1	

Results

Titanocene dichloride in the dosage of 3 x 30 mg/kg and 3 x 20 mg/kg brought about a significant reduction in tumour volume ($p<0.05$) compared to the control group (0.9% saline) and to the treatment group under medication with titanocene dichloride 1 x 30 mg/kg. There were no significant changes in body weight of nude mice (see Table 2). Concerning reduction of tumour volume increase the application of 3 x 10 mg/kg titanocene dichloride (T/C: +48.250%) was much more effective than the medication of 1 x 30 mg/kg titanocene dichloride (T/C: +126.552%) and even still more effective than the dosage of 1 x 40 mg/kg titanocene dichloride (T/C: +52.840%) (see Table 2).

Toxicicy-related deaths occurred in 2/8 animals (25%) of the 3 x 10 mg/kg titanocene dichloride group, in 2/8 animals (25%) of the 3 x 20 mg/kg titanocene dichloride group, 4/8 in animals (50%) of the 3 x 30 mg/kg titanocene dichloride group, in 2/9 animals (22.2%) of the 1 x 30 mg/kg titanocene group and in 3/9 animals (33.3%) of the 1 x 40 mg/kg titanocene dichloride group (see Table 2). Statistical significant differences in toxicity-related deaths in the treatment groups could not be observed.

Discussion

Testing of human tumours heterotransplanted to nude mice plays an important role in preclinical screening of cytostatic drugs since human tumours preserve drug susceptibility and histologic reactivity after xenografting them into nude mice [15, 16]. It thus seems possible to determine the activity of new cytostatic agents against human tumours as early as in the preclinical stage. Several studies have shown that a high correlation indeed exists between responses obtained with individual human tumours growing in nude mice and the clinical results with the same drugs [17, 18].

Although a number of drugs have been identified to have clinical activity against ovarian carcinoma, including platinum derivates, alkylating agents, anthracyclines and recen-

Table 2. — *Results of the chemotherapy trials*

Chemotherapy	$\Delta V\ t_x\text{-}t_0$ (mm^3)	$\Delta W\ t_x\text{-}t_0$ (g)	Slope factor α (g/day)	Slope factor β (mm^3/day)	T.C %	Toxic deaths (%)
titanocene 3 x 10 mg	+ 204.54	–2.1644	–0.3092	+29.22	+ 48.250	25
titanocene 3 x 20 mg	+155.82	–3.0142	–0.4306	+22.26	+ 36.757	25
titanocene 3 x 30 mg	+ 36.54	+1.0787	+0.1541	+ 5.22	+ 8.620	50
titanocene 1 x 30 mg	+536.48	+1.7787	+0.2541	+76.64	+126.552	22.2
titanocene 1 x 40 mg	+224	+4.004	+0.0572	+32.00	+ 52.840	33.3
saline	+423.92	–1.3048	–0.1864	+60.56		0

T/C (%) = Tumour volume (treated groups) x 100 / Tumour volume (control groups)

tly Paclitaxel, the majority of patients will still fail to be effectively treated by these standard therapeutic regimens, mostly due to acquired drug resistance [19, 20]. Organometallic complexes like titanocene dichloride represent a new class of chemicals that might feature meaningful antitumour activity and should be tested in ovarian cancer.

In the present study, different dosages of titanocene dichloride were used to explore the effect on human ovarian cancer tissue transplanted into nude mice. We found a significant reduction of tumour volume increase under medication of titanocene dichloride 3 x 20 mg/kg and 3 x 30 mg/kg compared to the application of titanocene dichloride 1 x 30 mg/kg without any significant differences in body weight changes of nude mice. Furthermore no statistical significant differences in toxicity-related deaths were found.

In other animal trials, titanocene reduced the size of treated colon 38 adenocarcinomas to less than half [21]. In this context, it is worth mentioning that in particular the colon 38 adenocarcinoma is rather insensitive to established cytostatics and is inhibited by few cytostatic drugs, e.g. by 5-fluoruracil or cyclophosphamide [22]. The stomach carcinoma M-Stg 4 was reduced in size by 73% or 60%. Under the influence of a higher dose of titanocene, two out of five tumours totally disappeared and never regrew. These results are remarkable since gastrointestinal carcinomas are generally rather insensitive to common cytostatic agents. The toxicity for organometallic bis-(cyclopentadienyl)-metal complexes differs fundamentally from the toxic characteristics of classical organic cytostatics, mainly damaging the proliferative activity of the bone marrow, and from those of organic platinum compounds, which mostly injure the structure and function of the kidneys at low therapeutic dose levels [12, 23]. This constellation is advantageous isofar as it should open up the possibility of combination therapy comprising metallocene complexes and organic and/or inorganic cytostatics without the danger that the toxic side-effects will be potentiated.

In animals treated with titanocene dichloride, a 10-fold reduced toxicity compared to cisplatin could be observed [7]. In human tumours grown in vitro or heterotransplanted to nude mice the anticancer efficacy of titanocene dichloride was similar or even better compared to cisplatin [5]. In studies using ovarian carcinoma cell lines which were selected for resistance against cisplatin or doxorubicin a lack of cross-resistance between titanocene dichloride and both cisplatin and doxorubicin could be observed [7]. DNA repair mechanisms are likely to influence the sensitivity of tumour cells against titanocene dichloride. The activity of titanocene dichloride in doxorubicin resistant ovarian cell lines does not suggest titanocene as a sustrate of P-glycoprotein [7]. Intranucleic mechanisms such as enhanced activity of repair enzymes are known to play an important role in platinum resistance by recovering drug-damaged DNA [24, 25]. Thus, immediate DNA complexation seems to determine the anticancer efficacy of biologically active titanocene dichloride [26].

Concerning the high activity of titanocene dichloride in vivo, this new compound could be an interesting agent in the treatment of a variety of human tumours including ovarian cancer. Titanocene dichloride has recently passed clinical phase I evaluation showing that nephrotoxicity and hepatotoxicity are of dose-limiting character and is currently in clinical phase II trials in patients with colorectal, breast and renal cell carcinomas [13, 14].

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