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 chemotherapy drug in women with ovarian carcinoma not responding to standard therapies.

Key words: Nude mouse; 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 mole-

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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-

lecules, 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-resistant tumour cells in vitro and in vivo [7]. Clinical phase I trials have been conducted showing that nephrotoxicity and hematotoxicity 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.
cimen. The tumour was cut into small fragments of about 20 mm² 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 cisplatin (100 mg/m²) and treosulfan (5 g/m²).

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 planned 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 (p/6 x length x width x height). Treatment was administered when the median tumour volume reached about 600 mm³.

Statistical Methods: All computations are based on average values from two implant sites. ΔWeight (ΔW t₁ - t₂) and ΔVolume (ΔV t₁ - t₂) were determined by the difference of body weights and tumour volumes at the end (Wt/Vt t₁) and at the beginning (Wt/Vt t₂) 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 slopes

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).

Toxicity-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, 2/9 animals (22.2%) of the 1 x 30 mg/kg titanocene dichloride 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-

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T/C (%) = Tumour volume (treated groups) x 100 / Tumour volume (control groups)
tly Paclitaxel, the majority of patients will still fail to be effec-
tively 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 anti-
tumour activity and should be tested in ovarian cancer.

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

In other animal trials, titanocene reduced the size of trea-
ted 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 cy-
tostatics and is inhibited by few cytostatic drugs, e.g. by 5-
fluorouracil or cyclophosphamide [22]. The stomach carci-
noma M-Stg 4 was reduced in size by 73% or 60%. Under
the influence of a higher dose of titanocene, two out of fi-
vour tumours totally disappeared and never regrew. These re-
sults 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 char-
acteristics of classical organic cytostatics, mainly damag-
ing 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 ther-
apeutic dose levels [12, 23]. This constellation is advanta-
geous isofar as it should open up the possibility of combi-
nation therapy comprising metallocene complexes and or-
ganic 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 di-
chloride was similar or even better compared to cisplatin
[5]. In studies using ovarian carcinoma cell lines which
were selected for resistance against cisplatin or doxorubi-
cin a lack of cross-resistance between titanocene dichlo-ide 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 resi-
stant ovarian cell lines does not suggest titanocene as a su-
strate of P-glycoprotein [7]. Intracellular mechanisms such as enhanced activity of repair enzymes are known to
play an important role in platinum resistance by recove-
ring 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 colo-
rectal, breast and renal cell carcinomas [13, 14].

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References
sis of surgery in advanced ovarian carcinoma: is maximum
cytoreductive surgery an independent determinant of pro-
agement of ovarian cancer”. In: Cancer of the ovary. Markman M., Hoskins W. J. eds., New York,
analysis of the role of platinum compounds in advanced
ovarian carcinoma”. The Advanced Ovarian Cancer Tru-
Sass G., Kreienberg R.: “Antitumour activity of new organ-
ometallic compounds in human ovarian cancer cell lines and
comparison to platinum derivatives”. *Anticancer Research,*
1997, 17, 815.
plexes: influence on xenografted human adenocarcinomas
of the gastrointestinal tract”. *Cancer Chemother. Pharma-
Sass G., Hübner H. et al.: “In vitro activity of titanocene-
dichloride in human renal cell carcinoma compared to con-
terventional antineoplastic agents”. *Anticancer Research,*
1994, 14, 1529.
Y.: “Titanocene-dichloride activity in cisplatin and doxorubi-
cin-resistant human ovarian carcinoma cell lines”. *Eur. J.
Cancer,* 1993, 29(7), 1000.
[8] Köpf-Maier P.: “Complexes of metals other than platinum as
[9] Kurbacher C. M., Bruckner H. W., Andreotti P. E., Kurba-
ccher J. A., Sass G., Krebs D.: “In vitro activity of titanoc-
enedichloride versus cisplatin in four ovarian cell lines
evaluated by microtiter plate bioluminescence assay”. *Anti-
cancer Drugs,* 1995, 6, 697.
itanocene dichloride a new organometallic compound,
active in vivo against various human tumours with dif-
ferential sensitivity to doxorubicin and cisplatin”. Proceed-
ings of the 86th Annual Meeting of the American Associa-
tion for Cancer Research, March 18-22, 1995, Toronto,
Ontario, Canada; Volume 36, 391.
xenografted sensitive human tumours after treatment with
the antitumour agent titanocene dichloride”. *J. Struct.
locene dichlorides: The effect of (Ch3)2TiCl3 and
(Ch3)2VCl3 on renal structure”. *Toxicol.,* 1986, 38, 81.
[13] Christodoulou C., Ferry D., Fyle D., Young A., Doran J.,
Sass G. et al.; “Phase I clinical trial of titanocene dichlori-
(de) with pharmacokinetic analysis”. Proceedings of the 88th Annual Meeting of the American Association
for Cancer Research. April 12-16, 1997; San Diego, CA;
Volume 38, 1495.


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