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Research paper

Human ovarian cancer xenografts in nude mice: chemotherapy trials with paclitaxel, cisplatin, vinorelbine and titanocene dichloride

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The new cytostatics titanocene dichloride and vinorelbine were compared to cisplatin and paclitaxel using a human ovarian cancer xenografts model. Biopsy material from a native human ovarian carcinoma was expanded and transplanted into 96 nude mice. The animals were divided into six treatment groups: cisplatin 3 × 4 mg/kg, paclitaxel 5 × 26 mg/kg, vinorelbine 1 × 20 mg/kg, titanocene dichloride 3 × 30 mg/kg, titanocene dichloride 3 × 40 mg/kg and a control group treated with 0.9% saline. Each experiment was repeated with eight mice in each treatment group. Treatment groups were evaluated in terms of average daily increase in tumor 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 tumor volume changes (β) within each group during the course of an experiment were calculated. Both a statistically significant decrease ($p<0.05$) in the body weight of the experimental animals (cisplatin: $\alpha=-0.5163$, vinorelbine: $\alpha=-0.6598$, paclitaxel: $\alpha=-0.6746$, titanocene dichloride 3 × 30 mg/kg: $\alpha=-0.6259$, titanocene dichloride 3 × 40 mg/kg: $\alpha=-0.7758$) and a significant reduction ($p<0.05$) of the increase in tumor volume (cisplatin: $\beta=12.049$, vinorelbine: $\beta=0.504$, paclitaxel: $\beta=-1.636$, titanocene dichloride 3 × 30 mg/kg: $\beta=6.212$, titanocene dichloride 3 × 40 mg/kg: $\beta=-0.685$) was shown in all treated groups compared to the control group ($\alpha=-0.1398$; $\beta=23.056$). No significant weight changes were observed between the individually treated groups. A statistically significant reduction of the tumor growth occured under paclitaxel ($\beta=-1.636$), vinorelbine ($\beta=0.504$) and titanocene dichloride medication 3 × 40 mg/kg ($\beta=-0.685$), as compared to the group treated with cisplatin ($\beta=12.049$). We found titanocene dichloride to be as effective as paclitaxel and more effective than cisplatin. Vinorelbine seems to be a very effective antineoplastic agent exhibiting a significant higher cytostatic effect than cisplatin. Both titanocene dichloride and vinorelbine provide new therapeutic options in women with ovarian carcinoma not responding to standard chemotherapy. [© 1998 Lippincott-Raven Publishers.]

Key words: Cisplatin, nude mice, paclitaxel, titanocene dichloride, tumor growth, vinorelbine.

Introduction

Ovarian carcinoma is a leading cause of deaths in gynecological cancer. First therapy of choice is R_0-R_1 cytoreduction followed by cisplatin chemotherapy. However, 5 years survival is only 20% due to the development of recurrent disease which often displays features of multidrug resistance against cisplatin and other chemotherapeutic agents like paclitaxel. Response to second-line therapy, at least in cisplatin-resistant disease, is worse.[1-9] New drugs—not cross-resistant to cisplatin and taxol—are urgently required.

Vinorelbine is a semisynthetic vinca alkaloid with cytostatic activity against a wide range of tumor cell lines.[10] It is a mitotic inhibitor believed to exert its antitumor effects by binding to tubulin, thus inhibiting microtubule assembly and eventually preventing metaphase tumor cell division.[11,12] The activity of deacetylvinorelbine, the main metabolite produced at a very low level, has the same activity as that of the parent drug. Both vinorelbine and its metabolite are mainly excreted via the bile.[13,14] Myelosuppression is the major dose-limiting toxicity of vinorelbine. Granulocytopenia occurs in more than 80% of individuals and is rated as moderate to severe in 30-40%.[15,16] Dosages used in clinical trials have ranged from 25 to 35 mg/m^2/week.

Titanocene dichloride is an early transition metal complex containing the intact bis(cyclopentadienyl)-titanium unit. The compound showed significant

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antitumor activity in a broad range of tumor models tested *in vivo* and *in vitro*.[17-23] 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. Bone marrow is usually not affected by the antiproliferative activity of titanocene.[24] Interestingly, titanocene dichloride is active against cisplatin-resistent tumor cells *in vitro* and *in vivo*.[19,20,22] Clinical phase I trials have been conducted showing that nephrotoxicity and hepatotoxicity are of dose-limiting character depending on the dosing regimen applied.[25,26] Titanocene dichloride is currently under clinical phase II evaluation in different human tumor entities.

In two different experiments with a total of 96 nude mice, we investigated the effect of different chemotherapeutics on tumor volume and body weight of the animals in order to appraise the efficacy and toxicity of the drugs.

Materials and methods

Chemotherapeutics

Titanocene dichloride was purchased from Medac (Hamburg, Germany), cisplatin from Rhone-Poulenc Rorer (Cologne, Germany), paclitaxel from Bristol-Myers Squibb (Princeton, NJ) and vinorelbine from Pierre Fabre (Boulogne, France).

Animals

Six-week-old athymic nude mice derived from an independent company (Harlan-Winkelmann, Borchen, Germany) were used for all experiments. Mice were maintained under barrier conditions and given sterilized food (Altromin, Lage, Germany) and water.

Heterotransplantation of tumor into nude mice

Human tumor tissue was freshly obtained from one patient suffering from advanced epithelial ovarian cancer. The tumor was cut into small fragments of about 20 mm^3 and implanted s.c. into both sides of back nude mice. Usually, no essential difference in tumor growth was observed between both implantation sides.

Characteristics of the primary tumor

Staging for ovarian cancer was carried out in accordance with the most recent FIGO classification. The primary tumor stage was FIGO IIIc. The histology showed a dedifferentiated serous ovarian adenocarcinoma with tumor 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/m^2) and treosulfan (5 g/m^2).

Procedures and design of the study

The protocol of the study was planned as described in Table 1. At 2 day intervals tumor volumes and animals' body weights were assessed. In the test groups, treatment was started when the median tumor volume reached about 600 mm^3. In several groups of both experiments, some mice died within the observation period of 17 days. All survivors were sacrificed at the end of each experiment. Tumors were measured in three perpendicular diameters and their volumes were

Table 1. Design of the study

Experiment	Group	*n*	Chemotherapy	Dose (mg/kg)	Day of application	Period of observation (days)
1, 2	1	8	cisplatin	3 × 4 mg	1, 3, 5	17
	2	8	vinorelbine	1 × 20 mg	1	
	3	8	paclitaxel	5 × 26 mg	1, 2, 3, 4, 5	
	4	8	titanocene	3 × 30 mg	1, 3, 5	
	5	8	titanocene	3 × 40 mg	1, 3, 5	
	control	8	0.9% saline		1	

estimated using the formula: $\pi/6 \times$ length $\times$ width $\times$ height.

Treatments

All treatments were administered i.p. In experiment 1 and 2, paclitaxel was given at a dose of 26 mg/kg, cisplatin at a dose of 4 mg/kg, titanocene dichloride at doses of 40 and 30 mg/kg, vinorelbine at a dose of 20 mg/kg every treatment day (see Table 1).

Statistical methods

All computations are based on average values from two implant sites. Δweight ($\Delta Wt_x - t_0$) and Δvolume ($\Delta Vt_x - t_0$) were determined by the differences of the body weights and tumor volumes at the end (Wt_x/Vt_x) and at the beginning (Wt_0/Vt_0) of the experiments. Since observation durations differed between groups and individuals (linear regression) treatment groups were evaluated by determining the average daily increase in tumor volume and average daily body weight increase of nude mice based on calculating slopes of least-square regressions performed on individual animals with slope factors α and β of the body weight (α) and tumor volume (β) changes within each group.

Statistical comparisons were based on analysis of variance (ANOVA). Univariate ANOVA was performed when comparing treatments within each experiment. Identical treatment groups from two different experiments were pooled and compared by two-factorial ANOVA.

Results

Cisplatin

No significant body weight change occured in experiment 1. We did not find any significant changes in tumor volumes compared to the control group in the two experiments. In experiment 2, a significant reduction in body weight ($p < 0.05$) of the experimental animals compared to the control group was observed during cisplatin administration (see Table 2; Figures 1 and 2).

Vinorelbine

Statistically significant body weight changes were not found in experiment 1; nevertheless, a very much greater absolute decrease in body weight was observed under vinorelbine administration. During vinorelbine treatment, a significant reduction in body weight ($p < 0.05$) of the experimental animals as compared to the control group occured only in experiment 2.

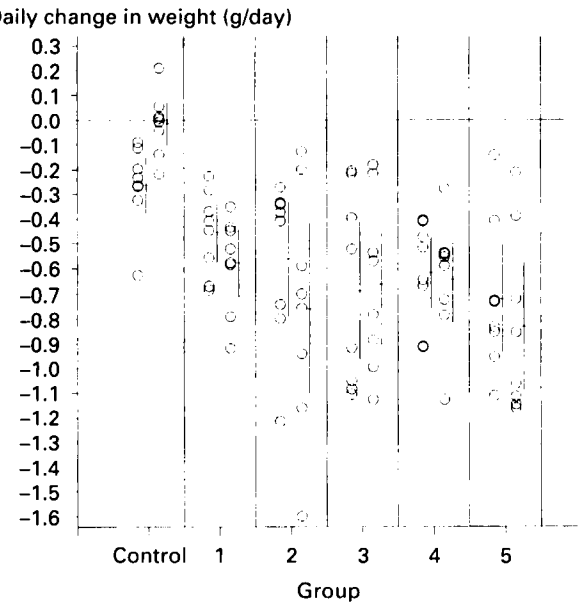

Figure 1. Data of body weight changes [calculated by slope factor α (g/day)] of the treatment groups (control: 0.9% saline; 1: cisplatin; 2: vinorelbine; 3: paclitaxel; 4: titanocene 3×30 mg/kg; 5: titanocene 3×40 mg/kg) in experiment 1 and 2 are presented in the form of box plots. The box depicts the first and third quartile. All observations are marked by circles. Lines are drawn from the extreme non-outlying observations to the box. The mean is marked by a vertical bar.

A significant change of tumor volumes ($p < 0.05$) under vinorelbine administration was observed in both experiments as compared to the control group and not as compared to the experimental animals treated with the other antineoplastic agents. In experiment 1, there was a very much lower absolute increase in tumor volume with vinorelbine administration than with cisplatin therapy and treatment with 3×30 mg/kg titanocene dichloride. The absolute increases in tumor volumes under 3×40 mg/kg titanocene dichloride therapy as well as under paclitaxel treatment were even lower. There was a similar situation in experiment 2 (see Table 2; Figures 1 and 2).

Paclitaxel

Compared to the control group, administration of paclitaxel resulted in a significant reduction in body

weight ($p < 0.05$) of the animals in experiment 1 and 2. A significantly lower increase in tumor volume under paclitaxel therapy was observed in experiment 1, both as compared to the control group and compared to the animals treated with cisplatin. A significant change ($p < 0.05$) in tumor volume occured only compared to the control group in experiment 2 (see Table 2; Figures 1 and 2).

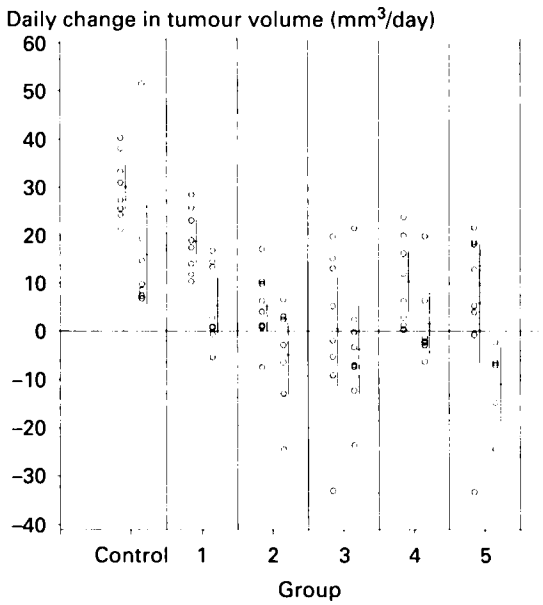

Figure 2. Data of tumor volume changes [calculated by slope factor β (mm^3/day)] of the treatment groups (control: 0.9% saline; 1: cisplatin; 2: vinorelbine; 3: paclitaxel; 4: titanocene 3 × 30 mg/kg; 5: titanocene 3 × 40 mg/kg) in experiment 1 and 2 are presented in the form of box plots. The box depicts the first and third quartile. All observations are marked by circles. Lines are drawn from the extreme non-outlying observations to the box. The mean is marked by a vertical bar.

Titanocene dichloride

Titanocene dichloride in the dosage 3 × 30 mg/kg produced a significant reduction in body weight ($p < 0.05$) compared to the control group in experiment 2. In experiment 1 there was a significant change in tumor volume during administration of 3 × 30 mg/kg titanocene dichloride compared to the control group. A significant change in nude mice body weight and in the tumor volume occured under medication with titanocene 3 × 40 mg/kg only as compared to the control group (see Table 2; Figures 1 and 2).

Two-factorial analysis of multivariance

The alterations in body weight and tumor volume in experiments 1 and 2 were compared by means of two-factorial analysis of multivariance. A statistically significant decrease in body weight ($p < 0.05$) of the experimental animals (cisplatin: $\alpha = -0.5163$, vinorelbine: $\alpha = -0.6598$, paclitaxel: $\alpha = -0.6746$, titanocene dichloride 3 × 30 mg/kg: $\alpha = -0.6259$, titanocene dichloride 3 × 40 mg/kg: $\alpha = -0.7758$) as well as significant changes in tumor volume (cisplatin:

Table 2. Results of the chemotherapy trials

Experiment	Chemotherapy	$\Delta Vt_x - t_0$ (mm^3)	$\Delta Wt_x - t_0$ (g)	Slope factor α (g/day)	Slope factor β (mm^3/day)	Toxic deaths (%)
1	titanocene 3 × 40 mg	98.056	−12.2298	−0.7194	5.768	37.5
	titanocene 3 × 30 mg	174.811	−10.4941	−0.6173	10.283	12.5
	cisplatin	318.274	−7.7384	−0.4552	18.722	0
	paclitaxel	8.126	−11.6926	−0.6878	0.478	75
	vinorelbine	89.42	−9.5302	−0.5606	5.260	37.5
	saline	512.312	−4.4931	−0.2643	30.136	0
2	titanocene 3 × 40 mg	−187.153	−14.1474	−0.8322	−11.009	37.5
	titanocene 3 × 30 mg	26.486	−10.9089	−0.6417	−1.558	25
	cisplatin	91.375	−9.8158	−0.5774	5.375	0
	paclitaxel	−63.767	−11.2438	−0.6614	−3.751	50
	vinorelbine	−83.81	−12.903	−0.7590	−4.930	25
	saline	271.592	−0.2618	−0.0154	15.976	0

β=12.049, vinorelbine: β=0.504, paclitaxel: β=−1.636, titanocene dichloride 3 × 30 mg/kg: β=6.212, titanocene dichloride 3 × 40 mg/kg: β=−0.685) were shown in all treated groups compared to the control group (body weight changes: α=−0.1398; change in tumor volume: β=23.056). Significant body weight changes were not observed when the individual treated groups were compared with each other. Compared to the cisplatin group (β=12.049) statistically significant alterations in tumor volumes ($p<0.05$) were seen with paclitaxel (β=−1.636), vinorelbine (β=0.504) and titanocene dichloride 3 × 40 mg/kg (β=−0.685).

Toxicity-related deaths

In experiment 1, 25% of nude mice died under titanocene dichloride, 75% under paclitaxel and 37.5% under vinorelbine. In experiment 2, 31.25% of nude mice died under titanocene dichloride, 50% under paclitaxel and 25% under vinorelbine. The total toxic death rate was: titanocene dichloride 28.125%, paclitaxel 62.5% and vinorelbine 31.25% (see Table 2).

Discussion

In the present study, different antineoplastic agents were used to explore the effect on human ovarian cancer tissue transplanted into nude mice. We found a significant reduction of tumor volume increase with paclitaxel, cisplatin, vinorelbine and titanocene dichloride compared to 0.9% saline. Paclitaxel or vinorelbine treatment seems to be more effective than a cisplatin treatment just as 3 × 40 mg titanocene dichloride causes a more highly significant reduction of tumor growth than cisplatin. In contrast to this, toxicity of paclitaxel, vinorelbine and titanocene dichloride indicated by body weight loss was comparable to that of cisplatin.

In other animal trials, titanocene dichloride reduced the size of colon 38 adenocarcinomas to less than 50%.[27] This is a worthwhile success since the colon 38 adenocarcinoma is rather insensitive to established cytostatics with the exception of 5-fluoruracil or cyclophosphamide.[28] Likewise, titanocene dichloride reduced the size of the heterotransplanted gastric carcinoma M-Stg 4 to 27–40% of the control. Using a higher dose of titanocene dichloride, two out of five tumors totally disappeared and never regrew. These results are remarkable since gastrointestinal carcinomas are generally rather resistant to common cytostatic agents. The toxicity of organometallic bis-(cyclopentadienyl)-metal complexes differs fundamentally from the toxic characteristics of both classical organic cytostatics, mainly damaging the proliferative activity of the bone marrow, and of organic platinum compounds, which mostly impair the renal structure and function at considerably low therapeutic dose levels.[24,29] This toxicity profile is advantageous in that it facilitates combination therapy comprising metallocene complexes and organic and/or inorganic cytostatics without potentiating toxic side effects. In the present study, we found titanocene to be as effective as paclitaxel and even more effective than cisplatin without any further side effects compared to paclitaxel (see toxic deaths). So far, titanocene dichloride has not been used for clinical treatment of ovarian cancer. Further studies are required to substantiate these results, but there are indications of a valuable chemotherapeutic action particularly in instances with acquired cisplatin resistance.[17,20] 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.[25,26]

Vinorelbine is a semisynthetic vinca alkaloid formed by modification of the catharanthine rather than the vindoline nucleus. This alteration was proposed to result in a different spectrum of experimental antitumor activity and toxicity for vinorelbine compared to other members of this class. The largest body of evidence pertaining to vinorelbine has been obtained in patients with non-small cell lung cancer and advanced breast cancer. Single-agent vinorelbine has produced objective response rates of 30–50% as first-line and about 15–30% as second-line chemotherapy for advanced breast cancer.[30,31] Combination chemotherapy incuding vinorelbine and other chemotherapeutic agents shows improved response rates comprising 55–75% for first-line and 30–50% for second-line therapy. In two studies improved quality of life was reported in 47% and relief of symptoms in 65% of women with advanced breast cancer receiving vinorelbine.[32–34] Ovarian cancer seems to be another indication in which vinorelbine might prove active. Evidence for the efficacy of vinorelbine in advanced ovarian cancer is confined mainly to results from two non-comparative trials with 20 and 50 women who could be evaluated.[35,36] All participants had relapsed or progressed on prior chemotherapy, primarily cisplatin/doxorubicin. In this platinum-refractory group, second-line or later strategy as a single agent produced a response rate of 14%.[35] Burger *et al.*[37] observed a response rate in four out of eight women (two complete and two partial remissions). Combining

vinorelbine with oral hexamethylmelamine, Pinel *et al.*[36] observed an increased response rate of 35% in 20 patients. Complete responses were obtained in 10% of patients, compared with 2% obtained in patients receiving single-agent vinorelbine. Furthermore, the results of phase II studies indicate a very encouraging activity in multiple myeloma and prostate cancer.

In the present study, vinorelbine was a very effective chemotherapeutic agent with a significantly improved cytotoxicity effect than cisplatin and it could be a helpful drug in women not responding to standard therapy.

Both vinorelbine and titanocene dichloride led to less manifest side effects in animals than paclitaxel. This would also increase the quality of life in humans if clinically confirmed. Further studies are required in order to validate titanocene dichloride with the objective of clinical application.

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