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Activity of Titanocene Dihalides against a Human Colon Carcinoma Heterotransplanted to Athymic Mice*

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Abstract—*The antitumor activity of* cis-*diamminedichloroplatinum(II) and of two metallocene derivatives, titanocene dichloride* $(C_5H_5)_2TiCl_2$ *and titanocene dibromide* $(C_5H_5)_2TiBr_2$, *was investigated against a human colon adenocarcinoma heterotransplanted to athymic mice. The substances were administered at various doses on a* Q2D×5 *or a* Q3D×5 *schedule. Whereas* cis-*diamminedichloroplatinum (II) induced an only marginal tumor-inhibiting effect, both titanocenes markedly suppressed tumor development (T/C values:* 23-40%) *and caused stagnation and relative decrease of tumor growth, when they were applied in subtoxic doses far below the* LD_{10} *level. The results are remarkable with respect to the general insensitivity of human colorectal carcinomas to cytostatic agents.*

INTRODUCTION

THE METALLOCENE dihalides $(C_5H_5)_2MX_2$ (M = Ti, V; X = F, Cl, Br, I, NCS, N_3) represent a group of organometallic complexes, the antineoplastic activity of which has been demonstrated against various animal tumor systems, e.g. Ehrlich ascites tumor, Lewis lung tumor or melanoma B16 [1-4; Köpf-Maier *et al.*, unpublished results]. Experimental attempts to reveal a possible anticancer activity of the complexes against human tumors have not yet been undertaken.

We therefore intended to investigate the cytostatic properties of two titanocene dihalides, $(C_5H_5)_2TiX_2$ with X = Cl or Br, against human tumors heterotransplanted to athymic, nude mice. The latter are appropriate hosts for human malignant tumors and, for that reason, offer the possibility of performing preclinical screening of new anticancer agents against human tumors and of evaluating the antineoplastic properties of substances against human malignancies under experimental *in vivo* conditions [5-7].

In the present study we report on the response of a human colon adenocarcinoma xenograft to administration of titanocene dihalides and, for comparitive purposes, also to treatment with the inorganic drug *cis*-diamminedichloroplatinum (II).

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MATERIALS AND METHODS

Substances

Both metallocenes titanocene dichloride $(C_5H_5)_2TiCl_2$ and titanocene dibromide $(C_5H_5)_2TiBr_2$, as well as the inorganic drug *cis*-diamminedichloroplatinum(II) (cisplatin, *cis*-$(NH_3)_2PtCl_2$), were prepared according to literature methods [8-11]. Elemental analyses (C, H, N) gave deviations $\leqslant$0.5% of the calculated values; no impurities were detectable by IR, NMR and mass spectra.

Animals

Male athymic mice (NMRI, nu/nu) were purchased from the Zentralinstitut für Versuchstierzucht, Hannover, F.R.G., and kept in laminar flow units. The cages, bedding, food and water were autoclaved before being placed in contact with the nude mice; Borgal® (Hoechst, Frankfurt, F.R.G.) was added to the drinking water to prevent infections. At the time of tumor inoculation, the animals were 8-10 weeks old and weighed about 18-20 g.

Tumor

The test tumor used in the present study was designated S90 and represented an adenocarcinoma deriving from the sigmoid colon. It was obtained from Dr J. Mattern, Deutsches Krebsforschungszentrum, Heidelberg, F.R.G., when it had been transplanted through eight passages of nude mice. After further transplantation in our laboratory, the experiments were performed when the tumor was in the 10th, 13th or 15th passage in nude mice.

Generally, the tumors were removed from donor animals when they had a size of about 1–2 cm^3; they were dissected and minced mechanically, mixed with two-fold volumes of Hanks' salts solution, supplemented with streptomycin (1 mg/ml) and penicillin (10^3 IU/ml), and pressed several times through injection needles with a diameter of 0.9 mm. Volumes of 0.3 ml of this tumor cell brei were then inoculated subcutaneously into the right flank of experimental animals; thereafter, the animals were randomized into treatment and control groups, each consisting of 3–5 mice; the day of tumor implant was defined as day 0. Tumor take was 90–95%. On day 10 after tumor implant, when the tumors within control and treated groups had a size of 400–800 mm^3, chemotherapy was initiated. The experiments were concluded on day 20 (schedule Q2DX5) or on day 25 (Q3DX5).

Testing procedure

The substances cisplatin (Pt), titanocene dichloride (Tc) and titanocene dibromide (Tb) were administered in equitoxic doses according to the following schedules:

(A) Schedule A corresponded to an LD_{10} regimen* in nude mice for all three substances: Q2DX5, 6 mg/kg (Pt), 30 mg/kg (Tc), 60 mg/kg (Tb).
(B) Schedule B administered half of the doses of schedule A every 2 days: Q2DX5, 3 mg/kg (Pt), 15 mg/kg (Tc), 30 mg/kg (Tb).
(C) Schedule C applied half of the doses of schedule A every 3 days: Q3DX5, 3 mg/kg (Pt), 15 mg/kg (Tc), 30 mg/kg (Tb).

In all cases the first substance injection was given on day 10. The cytostatics were applied intraperitoneally, dissolved in volumes of 0.35–0.4 ml of a DMSO/saline mixture (1/19, v/v). The control animals only received 0.4 ml of the DMSO/saline mixture without drug addition.

*The LD_{10} value was determined in a separate experiment whereby all three substances applied according to the given regimen caused the death of 2/20 animals within 8 days after the first substance injection.

Every 2 or 3 days, two perpendicular diameters (length a, breadth b) of the tumors were measured by use of a graduated caliper. The tumor volumes v were then calculated according to the formula $v = \frac{1}{2}\ a \times b^2$. The individual tumor volumes, measured on day 12 and on later days, were related to the tumor volumes of the same animals, which had been measured on day 10; by division of both values, the relative tumor volume, i.e. the increase of tumor volume within an individual animal, could be determined. Mean values of relative tumor volumes and standard deviations were then calculated within all experimental groups and summarized in Figs 1 and 2. Additionally, T/C values were obtained by the ratio

$$\frac{\text{mean relative tumor volume of treated tumors}}{\text{mean relative tumor volume of control tumors}} \times 100\ (\%)$$

on a given experimental day. By this calculation procedure the growth inhibition, which was induced by treatment, could be estimated.

RESULTS

The tumors of control animals were characterized by a progressive exponential growth pattern; within the experimental period between days 10 and 25 after tumor implant, the volumes of control tumors increased by factors of 6–20 (Figs 1 and 2).

The influence of treatment with low doses of the three substances under investigation according to schedule C (Q3DX5) or schedule B (Q2DX5) on tumor development is shown in Figs 1 and 2a. Whereas treatment with cisplatin apparently did not alter growth behavior of the tumors and did not suppress tumor growth to a remarkable extent (T/C values on day 25 and 20: 97 or 73% respectively), application of titanocene dichloride and titanocene dibromide inhibited tumor development and markedly reduced tumor growth compared to the controls (Fig. 1). The T/C ratios, which were attained on day 25 (Q3DX5) or on day 20 (Q2DX5) after application of low doses of titanocenes, amounted to 38 or 31% in the case of titanocene dichloride and to 40 or 28% in the case of titanocene dibromide. No deaths occurred and no symptoms of general toxicity were recognizable during and after treatment with the three substances according to schedules C or B.

Applying the substances in higher doses according to schedule A (Fig. 2b), which caused the death of 10% of treated animals due to substance toxicity (cf. Testing Procedure), distinct retardation of tumor growth could also be induced in surviving animals by application of cisplatin, resulting in a T/C value of 36% on day

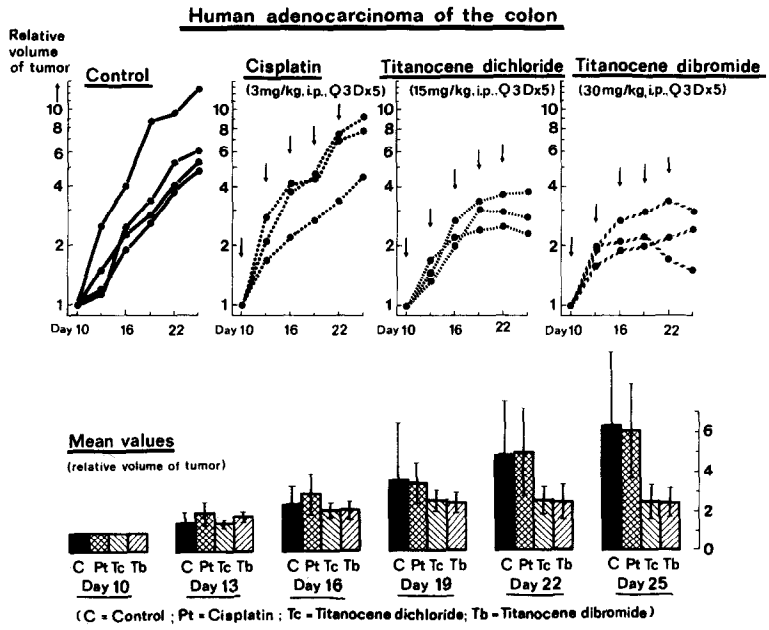

Fig. 1. Growth development of a human adenocarcinoma heterotransplanted to nude mice, under treatment with low doses of cisplatin, titanocene dichloride and titanocene dibromide administered on dose schedule C (Q3DX5). Upper part: growth curves of individual tumors; on abscissa, days after tumor implant on day 0; arrows indicate substance injections. Lower part: mean values of relative tumor volume and standard deviations within the control and treatment groups shown in the upper part.

20. For comparison, equitoxic doses of titanocene dichloride or titanocene dibromide, applied under the same conditions, also reduced tumor growth to a marked extent and caused T/C ratios of 23% (Tc) or 31% (Tb) on day 20. The following numbers of deaths occurred during the experimental period: 1/4 (Pt), 0/4 (Tc), 1/5 (Tb).

Analyzing the relation between the dose schedules administered and tumor development, a clear dependence of growth inhibition upon applied doses becomes apparent: increasing doses, applied within the experimental period by administration of the schedules C, B and A, resulted in final T/C values of 97, 73 and 36% in

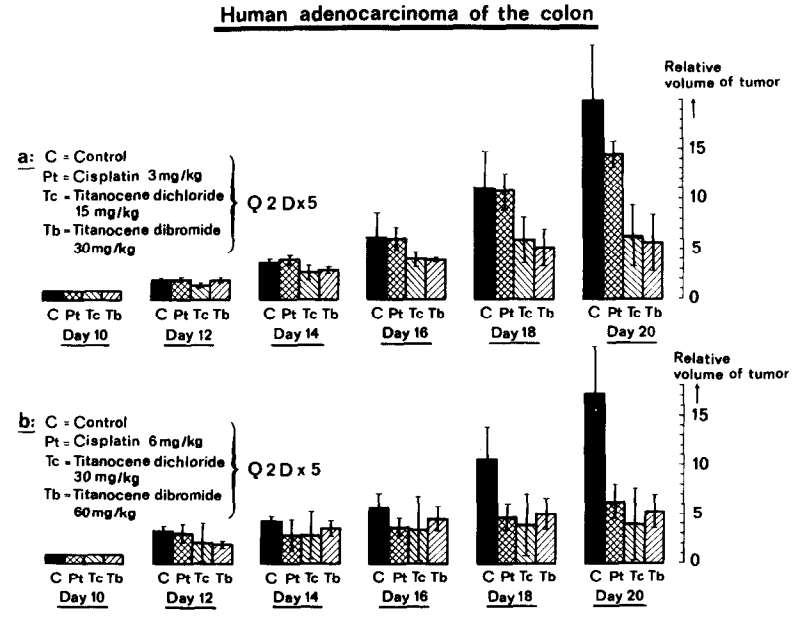

Fig. 2. Mean values of relative tumor volumes and standard deviations under treatment with low doses (Fig. 2a; schedule B) and high doses (Fig. 2b; schedule A) of cisplatin, titanocene dichloride and titanocene dibromide, administered on a Q2DX5 schedule; each column represents the mean value of 3-4 tumors.

the case of cisplatin, in T/C values of 38, 31 and 23% after application of titanocene dichloride and in T/C values of 40, 28 and 31% under the influence of titanocene dibromide.

DISCUSSION

Colorectal carcinomas represent neoplasms which are generally not very sensitive to established chemotherapeutic agents; only 5-fluorouracil and mitomycin-C have shown some activity against colon carcinomas [12, 13]; the effects achieved, however, are of little value with respect to patient survival. This apparent insensitivity of colorectal tumors to commonly used cytostatics has been confirmed during the last years by experimental results from *in vitro* investigations using the human tumor stem cell assay [14, 15] as well as by *in vivo* studies carried out in immune-deficient mice [6, 16]. The latter have been approved as hosts for heterotransplanted human malignant tumors and as model systems for performing preclinical therapeutic assays. Most authors agree that there is a high correlation between human tumor xenograft response to chemotherapy and clinical results with the same drugs [6, 7, 16, 17].

In the present study we investigated the effectiveness of some new inorganic and organometallic cytostatic agents against a human adenocarcinoma of the colon heterotransplanted to athymic, nude mice. It could be shown that, on the one hand, the newly developed inorganic drug *cis*-diamminedichloroplatinum(II), which is clinically characterized by marked antineoplastic activity against urogenital tumors as well as against carcinomas of the head and neck [18], failed to show a more than marginal antitumor effect against the colorectal carcinoma used in the present study and that it only inhibited tumor growth when it was applied in doses of the LD_{10} range; this finding is in accord with clinical experience [18, 19] and with results of other experimental studies performed in immune-deficient mice [16, 20]. On the other hand, the present study could demonstrate that the two metallocenes titanocene dichloride and titanocene dibromide caused a pronounced growth delay and a decrease in relative tumor volume when they were applied in subtoxic doses far below the LD_{10} level. This antiproliferative effect induced by titanocenes was revealed to be clearly dose-dependent and was much more pronounced than the growth-inhibiting activity of *cis*-diammine-dichloroplatinum(II) against the tumor used in the present study.

Since current clinical chemotherapy of malignant colorectal carcinomas generally gives only poor results, the findings of the present study are of interest with respect to the growth-inhibiting activity of titanocenes against a human colon carcinoma xenograft. However, further, extensive studies using a greater number of colorectal carcinomas heterotransplanted to nude mice are necessary to confirm this finding and to evaluate the actual antineoplastic effectiveness of titanocenes against colon carcinomas.

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