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Tumor inhibition by titanocene complexes: influence on xenografted human adenocarcinomas of the gastrointestinal tract

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Summary. The present study deals with the influence of some bis(η^5-cyclopentadienyl)titanium(IV) (titanocene) complexes, mainly represented by titanocene dichloride, on the development of several human gastrointestinal (GI) carcinomas (one stomach, seven colon, four sigmoid, and two rectal adenocarcinomas), all xenografted to athymic mice. In 10 of these 14 carcinomas, titanocene dichloride effected growth suppression of $>50\%$ in comparison with control tumors. In the case of the stomach and two colon adenocarcinomas, absolute decreases in tumor volume occurred during and after the treatment period, resulting in growth delays of 6, 14, and 31 days, respectively. No sensitivity dependence was observed in the degree of tumor differentiation. The findings of the present study confirm the tumor-inhibiting activity of titanocene complexes against human GI adenocarcinomas. These results are noteworthy in view of previous clinical and experimental experience indicating that human adenocarcinomas of the stomach and colon are generally rather insensitive to common cytostatic agents.

Introduction

In antitumor studies carried out in recent years, it has been shown that some organometallic bis(η^5-cyclopentadienyltitanium(IV) (titanocene) complexes are distinguished by antiproliferative properties against numerous animal tumors, especially Ehrlich ascites tumor, sarcoma 180, and diverse experimental solid tumors such as B16 melanoma, Lewis lung tumor, and colon 38 adenocarcinoma [9–12, 15]. Further pilot investigations of human tumors heterotransplanted to athymic mice have revealed some activity of titanocene compounds against single human carcinomas, e.g., two lung carcinomas [7] and a colon adenocarcinoma [13], whereby the latter tumor exhibited the typical pharmacologic behavior of colon carcinomas unresponsive to common cytostatic agents such as cyclophosphamide or cisplatin.

Human tumors heterotransplanted to athymic mice are comparable with tumors in individual patients [1, 5]; they do not represent standard tumor lines as do most experimental animal tumors. Consequently, a greater number of individual tumors of a given type of human tumor must be examined to determine the latter's sensitivity or insensitivity to the cytostatic agent under investigation. Therefore, in the present study we established several adenocarcinomas derived from the GI tract as serially transplantable xenografts in nude mice and analyzed their growth behavior in response to treatment with titanocene complexes.

Materials and methods

Antitumor agents. Some titanocene complexes (Fig. 1), represented by titanocene dichloride [$(C_5H_5)_2TiCl_2$], titanocene dibromide [$(C_5H_5)_2TiBr_2$], and titanocene bis(hydrogenmaleinate) [$(C_5H_5)_2Ti(cis\text{-}OOCCH=CHCOOH)_2$], were tested in the present study. The compounds were prepared and purified according to methods described in the literature [4, 14, 21, 23]. Elemental analyses (C, H, Ti) gave deviations of $\leq 0.5\%$ of the calculated values. Nuclear magnetic resonance (NMR), IR, and mass spectral characterization of the compounds revealed no evidence of impurities.

Animals. Male athymic mice (NMRI, nu/nu), purchased from Bomholtgard Breeding and Research Centre Ltd. (Ry, Denmark), were kept in isolators or laminar air-flow benches. Bedding, food, and water were autoclaved before being placed in contact with the animals. The drinking water was adjusted to pH 2.5 by the addition of hydrochloric acid. Antibiotics were not given. At the time of tumor transplantation, the animals were about 8–12 weeks old and weighed 18–22 g.

Tumors. Fourteen human GI carcinomas serially heterotransplanted to athymic mice were tested in the present study. Their origin and some of their histopathologic characteristics are summarized in Table 1. For propagation and testing purposes, when they reached a size of about 6–8 cm^3 the tumors were removed from donor animals, minced mechanically, pressed through injection needles, and suspended in 2-fold volumes of Hank's salt solution. Volumes of 0.3 ml tumor suspension were then injected s.c. into the right flank of athymic mice. Thereafter, the animals were randomized into control and treated groups, each group consisting of 3–6 animals. The day of tumor inoculation was defined as day 0 of the experiment.

Testing procedure. Chemotherapy was initiated when the tumors reached a size of 0.4–0.8 cm^3, which was attained between days 8 and 25 after tumor transplantation, depending on the growth of individual tumors. The com-

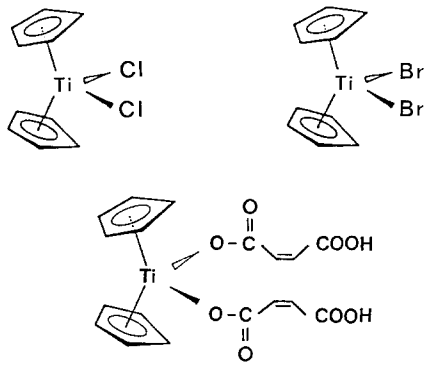

Fig. 1. Molecular structures of titanocene dichloride, titanocene dibromide and titanocene bis(hydrogenmaleinate)

Table 1. Characteristics of the GI tumors tested

Designation	Origin	Histopathology	Passage number
CX1[a]	colon	well-differentiated adenocarcinoma	–[e]
CX2[a]	colon	adenocarcinoma	–[e]
C-Stg2	colon	moderately differentiated adenocarcinoma	5–7
C-Stg3	colon	poorly to moderately differentiated adenocarcinoma	4–9
C-Stg6	colon	poorly to moderately differentiated adenocarcinoma	3–5
C-Hbg1[b]	colon	adenocarcinoma	31–35
C-Hbg2[b]	colon	adenocarcinoma	–[e]
S90[c]	sigmoid colon	adenocarcinoma	–[e]
S-Sb1	sigmoid colon	moderately differentiated adenocarcinoma	5–7
S-Sb2	sigmoid colon	moderately differentiated adenocarcinoma	3–8
S-Stg4	sigmoid colon	adenocarcinoma	4–7
R-Sb1	rectum	moderately differentiated adenocarcinoma	3–5
R85[d]	rectum	adenocarcinoma	12–17
M-Stg4	stomach	poorly differentiated adenocarcinoma	3–5

[a] Xenograft obtained from the National Cancer Institute (Bethesda, Maryland, USA)
[b] Xenograft obtained from Dr. U. Otto (Hamburg-Eppendorf, FRG)
[c] Xenograft obtained from Dr. H. H. Fiebig (Freiburg, FRG)
[d] Xenograft obtained from Dr. J. Mattern (Heidelberg, FRG)
[e] Passage number unknown

pounds were injected i.p. as 5-fold injections according to a schedule of either Q2D × 5 (every 2 days × 5) or Q3D × 5 (every 3 days × 5). The different doses given are listed in Tables 2 and 3, whereby those corresponding to LD_{10} or LD_{20} are labelled. Before drug administration, the compounds were dissolved in a mixture of dimethyl sulfoxide and saline (1/19, v/v) and injected i.p. in volumes of 0.4–0.5 ml (0.02 ml/g body weight). Control animals received 0.4 ml dimethyl sulfoxide/saline mixture only. Drug-induced toxic deaths were defined as deaths occurring within 10 days after the last drug injection.

On the days of treatment, i.e., every 2 or 3 days, the animals were weighed and two perpendicular diameters (length a, breadth b) of the tumors were measured with a graduated caliper. Absolute tumor volumes were calculated according to the formula $v = a \times b^2/2$. The measurement of tumors was continued beyond the last day of injection for a further 17–40 days, depending on the growth of individual tumors. Relative tumor volumes, indicating the increases in volume of individual tumors, were calculated by relating the absolute tumor volumes measured beginning on the 2nd day of treatment to that determined on the 1st day of drug injection. Within all experimental and control groups, mean values of relative tumor volume and standard deviations were then calculated for the different experimental days. Treated/control (T/C) values were obtained using the ratio

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

Results

Titanocene dichloride was tested against a total of 14 GI adenocarcinomas, including 7 colon carcinomas of different degrees of differentiation, 4 adenocarcinomas of the sigmoid colon, 2 rectal adenocarcinomas, and 1 poorly differentiated stomach adenocarcinoma (Table 1), whereas titanocene dibromide and titanocene bis(hydrogenmaleinate) were investigated against only 4 of the 14 tumors. The results of antitumor testing are documented in Tables 2 and 3, in which the T/C ratios attained on key dates are listed.

In the colon tumor xenografts CX1 and CX2, used as screening systems in the National Cancer Institute, tumor growth was suppressed by titanocene dichloride by 60%–70% (Table 2) at corresponding T/C ratios of 30%–40%. Figure 3 illustrates the clearly dose-dependent antiproliferative effect in the CX1 xenograft, resulting in growth delays of 7 and 14 days after the injection of 5 × 15 or 5 × 20 mg/kg titanocene dichloride, respectively. Another tumor responding to titanocene dichloride was the moderately differentiated colon carcinoma C-Stg2. Its reaction was characterized by absolute diminutions in tumor volume to 70% and 40% of the starting value from day 6 of the treatment period until day 14 (Fig. 4) and by dose-dependent growth delays of 13 and 31 days following the injection of 5 × 10 or 5 × 15 mg/kg titanocene dichloride, respectively. The T/C ratios determined at the end of the treatment period amounted to < 10%, corresponding to growth inhibitions of more than 90%. These effects were stable, lasting several weeks beyond the end of the treatment period. In investigating the behavior of the poorly to moderately differentiated colon adenocarcinoma C-Stg3 in response to treatment with titanocene dichloride, less pronounced yet significant growth inhibition of 60%–70% were induced. The growth of the C-Hbg1 xenograft was suppressed by scarcely 50%, whereas the proliferation behavior of tumors C-Stg6 and C-Hbg2 was obviously not influenced by titanocene dichloride (Table 2).

Table 2. Growth inhibition[a] effected by titanocene dichloride in diverse xenografted human GI carcinomas

Schedule	Dose (mg/kg)	CX1		CX2		C-Stg2		C-Stg3		C-Stg6		C-Hbg1		C-Hbg2	
		3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]
Q3Dx5	10	49	42	11	43	–	–	18	0	10	37	0	0	6	26
	15	57	70	38	52	–	–	71	66	22	39	0	0	6	6
	20	68	65	74	70	–	–	–	–	–	–	20	22	27	22
Q2Dx5	10	15	24	23	20	71	71	33	51	–	–	16	32	0	0
	15	41	47	38	33	91	94	60	58	–	–	49	41	0	0
	20[c]	68	61	–	–	87	92	66	78	–	–	44	58	0	18

Schedule	Dose (mg/kg)	S90		S-Sb1		S-Sb2		S-Stg4		R-Sb1		R85		M-Stg4	
		3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]
Q3Dx5	10	–	–	–	–	–	–	57	61	0	0	33	25	48	63
	15	64	60	–	–	–	–	48	69	7	2	55	53	33	23
	20	–	–	–	–	–	–	65	74	0	13	–	–	59	65
Q2Dx5	10	11	23	0	19	47	52	24	49	–	–	43	36	47	49
	15	67	64	42	52	52	48	71	83	–	–	51	55	69	76
	20[c]	75	71	58	55	49	47	–	–	–	–	–	–	58	72

[a] The parameter evaluated is tumor growth inhibition (in %) of control tumor size, calculated by 100% – T/C
[b] The values for tumor growth inhibition were determined 3 and 17 days after the last drug injection
[c] Regimens correspond to LD_{10} – LD_{20} doses

Table 3. Growth inhibition[a] effected by titanocene dibromide (TDB) and titanocene bis(hydrogenmaleinate) (THM) in four xenografted human colorectal carcinomas

Compound	Schedule	Dose (mg/kg)	S90		S-Sb1		S-Sb2		R85	
			3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]	3d[b]	17d[b]
TDB	Q3Dx5	10	–	–	–	–	24	41	0	0
		20	57	58	–	–	34	51	35	0
	Q2Dx5	10	2	18	19	27	–	–	0	0
		20	68	65	22	42	–	–	12	0
		40[c]	65	69	–	–	–	–	–	–
THM	Q3Dx5	30	28	35	46	59	–	–	51	50
		40[c]	59	42	–	–	–	–	–	–
	Q2Dx5	20	11	30	–	–	–	–	–	–
		30[c]	76	59	–	–	–	–	–	–

[a, b, c] See footnotes to Table 2

In carcinomas derived from the sigmoid colon, titanocene dichloride again effected growth suppressions of 60%–80% in the S90 and S-Stg4 tumors, whereas the proliferation of the moderately differentiated carcinomas S-Sb1 and S-Sb2 was reduced by only about 50% (Table 2). A similar effect was observed in the rectal carcinoma R85, whereas the rectal adenocarcinoma R-Sb1 was obviously not at all affected by titanocene dichloride.

The only tumor derived from elsewhere than the colon was the poorly differentiated stomach adenocarcinoma M-Stg4, which was obviously rather sensitive to the action of titanocene dichloride, its growth being inhibited by 60%–75% (Table 2) at T/C ratios of 25%–40%. About half of the tumors absolutely diminished in size during treatment with 5×10 mg/kg (Fig. 2) and were delayed in growth by 3 days. On treatment with 5×15 mg/kg, two of five tumors totally disappeared (Fig. 2). The mean growth delay of this treated group in comparison with the control group amounted to 6 days.

Pilot experiments with the related complexes titanocene dibromide and titanocene bis(hydrogenmaleinate) against selected colorectal carcinomas revealed antiproliferative activity similar to that observed for titanocene dichloride against the sigmoid adenocarcinoma S90 and less pronounced activity against the xenografts S-Sb1, S-Sb2, and R85 (Table 3). For purposes of comparison, cisplatin and 5-fluorouracil, one of the clinically approved cytostatic drugs against colorectal carcinomas, were given at equitoxic doses (1/2 LD_{10}, 3/4 LD_{10}, LD_{10}) to animals bearing the R85 or S90 xenografts. Neither of these compounds could induce more pronounced effects than the titanocene complexes; at the LD_{10} doses, both compounds caused

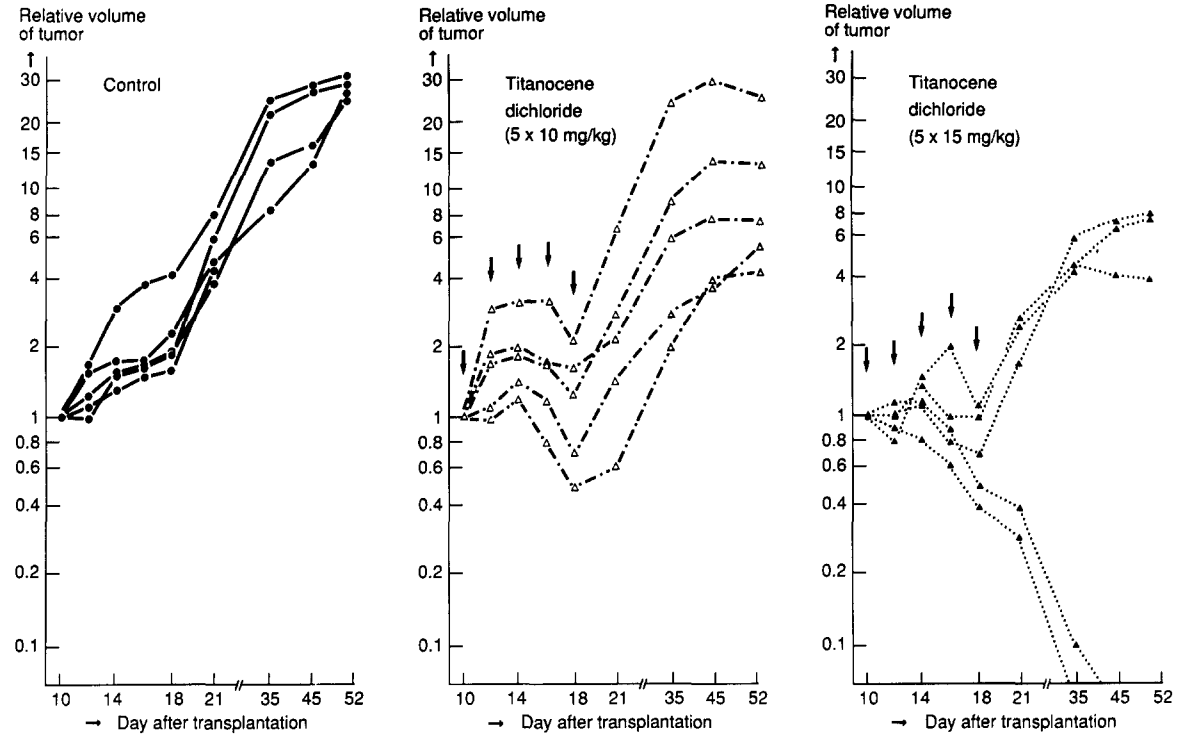

Fig. 2. Growth behavior of a xenografted human stomach adenocarcinoma (M-Stg4) under treatment with titanocene dichloride given at sublethal doses according to Q2D × 5 (D = 10 or 15 mg/kg) on days 10, 12, 14, 16, and 18 after tumor transplantation. The graphs represent growth curves of individual tumors. On the abscissa, days after the tumor implant on day 0. *Arrows* indicate drug injections

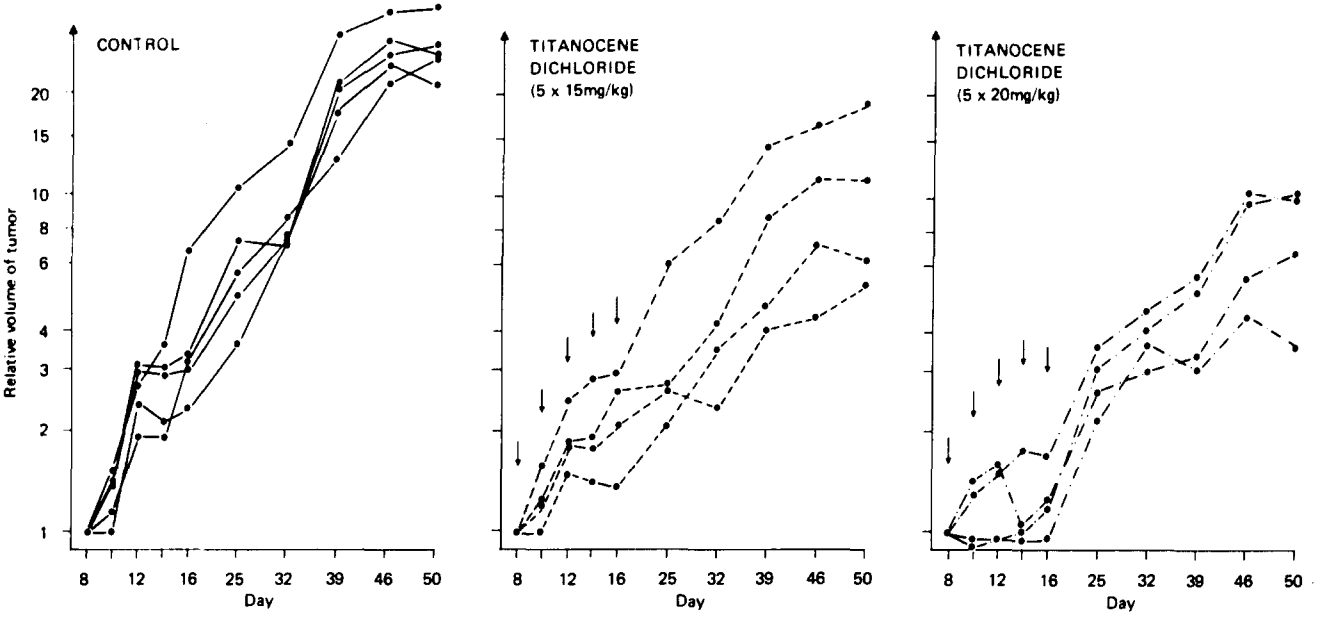

Fig. 3. Growth behavior of a xenografted human colon adenocarcinoma (CX1) under treatment with titanocene dichloride given according to Q2D × 5 (D = 15 or 20 mg/kg) on days 8, 10, 12, 14, and 16 after tumor transplantation (for further explanations, cf. legend to Fig. 2)

marginal activity and slowed down tumor proliferation by 20%–35% at corresponding T/C ratios ranging between 80% and 65%.

Discussion

Because of the congenital deficiency of a thymus and, consequently, the lack of competent T-lymphocytes in nude animals, xenografted malignant tumors proliferate in athymic animals in a manner comparable with metastases outside of human patients [19, 20] and thus are accessible to various experimental investigations. As the chemosensitivity of tumor tissue is retained after heterotransplantation, the nude animal model is apparently suitable for preclinical testing of the spectrum of activity of newly

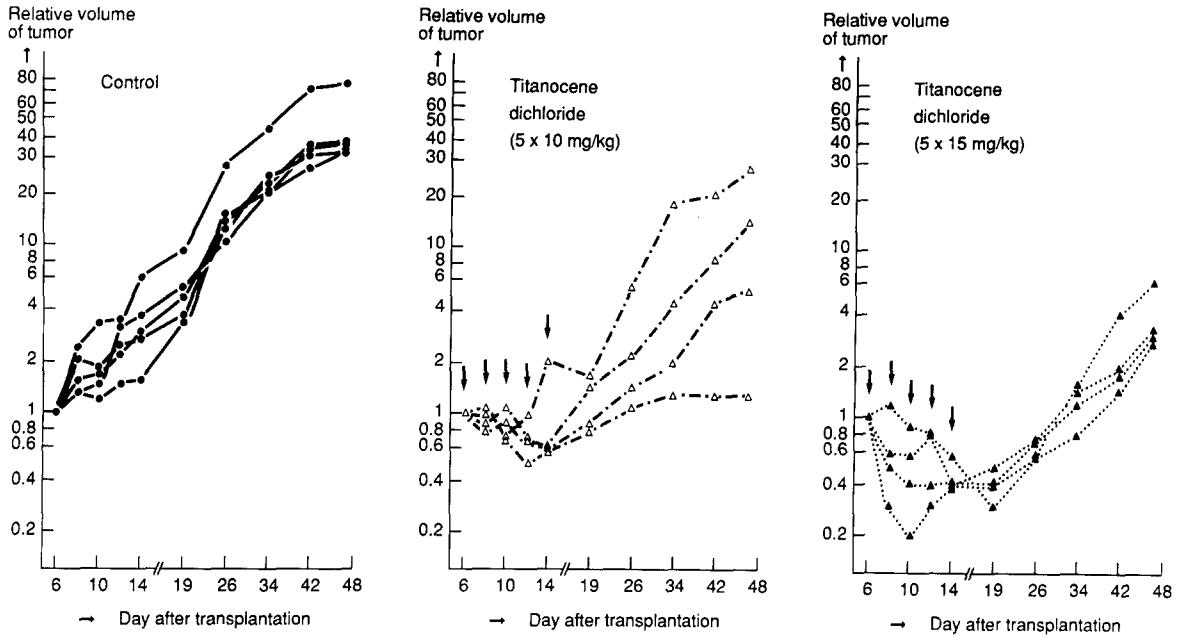

Fig. 4. Growth behavior of a xenografted human colon adenocarcinoma (C-Stg2) under treatment with titanocene dichloride given according to Q2D × 5 (D = 10 or 15 mg/kg) on days 6, 8, 10, 12, and 14 after tumor transplantation (for further explanations, cf. legend to Fig. 2)

developed cytostatic agents [2, 6, 22]. The predictive therapeutic value for human patients seems to be high, with the probability of correct predictions of tumor response or resistance ranging between 92% and 97% [6].

In agreement with this apparent imitation of the clinical situation in nude mouse experiments, human colon adenocarcinomas xenografted into athymic animals are generally insensitive to common cytostatic agents [17, 18] and only marginally respond to a few substances, e.g., 5-fluorouracil and mitomycin C [3, 16]. In view of the analogous behavior of tumors S90 and R85 in the present study, the finding that the growth of 10 of 14 GI tumors was suppressed by titanocene dichloride by >50% of the control tumor size must be considered a strong indication of the cytostatic properties of titanocene complexes, especially titanocene dichloride, against human GI adenocarcinomas.

Two other experimental observations underline the quite unusual antitumor activity observed with titanocene dichloride. First, the growth of the animal colon 38 adenocarcinoma, which also represents a rather insensitive experimental tumor [24], is inhibited by titanocene dichloride [9] to a similar extent as by 5-fluorouracil. Second, the analysis of morphologic alterations occurring in a heterotransplanted human colon adenocarcinoma following treatment with titanocene dichloride [8] has revealed profound nuclear and cytoplasmic changes within tumor cells, leading to the death of most heterotransplanted tumor cells within a few days. Thereafter, host-supplied inflammatory cells immigrated into the xenografts to remove the cellular debris [8].

Future investigations will show (a) whether the growth-suppressing activity of titanocene dichloride observed in the two heterotransplanted human lung carcinomas can also be confirmed in a greater number of xenografts, and (b) which other human tumors will respond to this newly developed, organometallic, early-transition metal complex.

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