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(\(\eta^5\)-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(\(\eta^5\)-cyclopentadienyl)titanium(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\(_5\)H\(_5\))\(_2\)TiCl\(_2\)], titanocene dibromide [(C\(_5\)H\(_5\))\(_2\)TiBr\(_2\)], and titanocene bis(hydrogenmaleinate) [(C\(_5\)H\(_5\))\(_2\)Ti(cis-OOCCH\(_\equiv\)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 < 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-
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 \times 15 or 5 \times 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 \times 10 or 5 \times 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).