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Antitumour activity of [1,2-di(cyclopentadienyl)-1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride in xenografted Ehrlich's ascites tumour

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Abstract

The effects of a new titanocene compound with an ansa ligand in the cyclopentadienyl rings, the 1,2-di(cyclopentadienyl)-1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride (TITANOCENE X), on the growth and differentiation of granulocyte–macrophage progenitor cells [colony-forming unit-granulocyte–macrophage (CFU-GM)] and Natural killer (NK) cell activity in Ehrlich's ascites tumour (EAT)-bearing mice were studied. Myelosuppression concomitant with increased numbers of spleen CFU-GM was observed in tumour-bearing mice. Treatment of these animals with TITANOCENE X (2.5–50 mg/kg/day) produced an increase in myelopoiesis, in a dose-dependent manner, and reduced spleen colony formation. In addition, the treatment of EAT-bearing mice with 3 doses of 20 or 50 mg/kg TITANOCENE X restored to normal values the reduced Natural killer cell function observed during tumour growth. In parallel, TITANOCENE X prolonged, in a dose-dependent manner, the survival of mice inoculated with Ehrlich's ascites tumour. The highest dose of 50 mg/kg prolonged in 50% the survival time of EAT-bearing mice, compared to non-treated tumour-bearing controls. In comparison with previous results from our laboratory addressing the effects of titanocenes on haematopoiesis, we observed with TITANOCENE X a similar effective profile as for bis(cyclopentadienyl) dithiocyanate titanium(IV), being both less effective than di(cyclopentadienyl) dichloro titanium(IV), since the latter not only prolonged, but also increased the rate of survival. These differences in efficacy may be due to the nature of the *ansa*-cyclopentadienyl ligand used in TITANOCENE X, since the C_2 bridge between the two cyclopentadienyl groups will increase the hydrolytic stability by an organometallic chelate effect. Also, the introduction of two dimethylamino substituents increases the water solubility of TITANOCENE X when compared to titanocene dichloride itself.

Keywords: Titanocene; Haematopoiesis; Tumour; Natural killer

1. Introduction

The formal use of metal complexes in chemotherapy can be traced back to 1969 when *cis*-diamminedichloroplatinum(II), *cis*-platin, showed to possess antitumour properties (Rosenberg et al., 1969). Subsequently, in the early seventies and eighties, platinum complexes became very popular antineoplastic agents with high efficacy against human testicular, ovarian, bladder, head and neck carcinomas (De Vita et al., 1985; Lippard and Pil, 1997; Wong and Giandomenico, 1999). However, toxic effects such as nephrotoxicity and myelotoxicity are the major drawbacks of this platinum complex for clinical applications (Caruso and Rossi, 2004).

The metallocene dichlorides $[(C_5H_5)_2MCl_2]$ with M = titanium, vanadium or molybdenum as central metal atom were the first early transition metal compounds with detected antitumour activity (Mokdsi and Harding, 2001). These substances exhibit marked antineoplastic activity against fluid and solid Ehrlich's

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tumour, fluid and solid Sarcoma 180, B16 melanoma, Lewis Lung carcinoma, mouse mammary tumour TA3Ha and colon 38 adenocarcinoma (Köpf-Maier, 1988; Valadares et al., 1998, 2003, 2004; Valadares and Queiroz, 2002,) and markedly inhibit the growth of xenografted human carcinomas of lung, breast, gastrointestinal tract and renal cell (Köpf-Maier, 1989, 1999). Among their appealing properties is that they do not show common side effects of widely used cytostatic agents such as emesis, alopecia or bone marrow impairment (Caruso and Rossi, 2004; Valadares et al., 1998; Valadares and Queiroz, 2002).

The exact mechanism of action for Titanocene compounds is still under discussion. It has been proposed that these species interact with DNA, inhibiting the cell cycle (Vera et al., 2004). However, the antitumoural mechanism of the titanocenes is most likely a complex pathway, probably involving a number of different biological molecules related to the transport and delivery of Ti species into cancer cells, and, after hydrolysis, subsequent interaction with nucleic acids and/or proteins and/or other potential coordinating constituents present in the intracellular environment (Mokdsi and Harding, 2001). The tendency to hydrolyse seems to be one of the hypotheses for the tumour-inhibiting potency of the titanocene dihalides. In this sense, we have recently demonstrated, using two different titanocenes, the di(cyclopentadienyl) dichloro titanium(IV) (DDCT) and its derivative, bis(cyclopentadienyl) dithiocyanate titanium(IV) (BCDT), that the increased hydrolytic stability produced by the substitution of halide by pseudohalide in the general formula of DDCT leads to a less therapeutically effective antitumoural compound (Valadares and Queiroz, 2002; Valadares et al., 2003, 2004). In these papers, we reported a more effective response of DDCT, as compared to BCDT, in the recovery of the myelosuppression and natural killer cell activity and in the prevention of the Th1→Th2 switch produced during tumour evolution. These features make titanium compounds interesting for combined therapy, supplementing the tumouricidal effect without myelotoxicity, and further studies for structure–activity relationship (Caruso and Rossi, 2004).

Although, titanocene dichloride reached clinical trials, very little synthetic effort was employed to increase the antitumoural effect of any titanocene dichloride derivative, despite novel methods starting from titanium dichloride or calcium and fulvenes (Eisch et al., 1998; Kane et al., 1997) allowing direct access to highly substituted *ansa*-titanocenes (Fox et al., 2002; Tacke et al., 2004a,b; Rehmann et al., 2005a,b). To further investigate the influence of structural changes in the therapeutic response of the titanocenes, a new compound with an ansa ligand in the cyclopentadienyl rings, the [1,2-di(cyclopentadienyl)-1,2-di(*p*-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride (TITANOCENE X), was introduced. In this compound, the C2 bridge between the two cyclopentadienyl groups in the ansa ligand increases the hydrolytic stability by an organometallic chelate effect. Controversial results are found in the literature related to chemical modification of the cyclopentadienyl rings and antitumoural activity. Köpf-Maier et al. (1981), using the Ehrlich's ascites tumour cells, pointed out that chemical modification of the cyclopentadienyl rings reduced antitumour activity. On the other hand, Boyles et al. (2001) observed increased effectiveness in vitro through the introduction of the electron withdrawing carbomethoxy group into the cyclopentadienyl rings.

Based on these findings, this work was designed to investigate the in vivo antitumour activity of TITANOCENE X, using the Ehrlich ascites tumour (EAT) experimental model. In previous work, we have demonstrated that the myeloproliferative properties of several antitumoural compounds are partially responsible for their antitumour activity in the EAT model. In this regard, we have investigated the effect of this new compound on the production of granulocyte–macrophage progenitor cells [colony-forming unit-granulocyte–macrophage (CFU-GM)] of EAT-bearing mice in parallel to the evaluation of survival rate. In addition, we also studied in these animals the total and the differential bone marrow cell counts and the activity of natural killer (NK) cells.

2. Material and methods

2.1. Mice

The mice used in this study were bred at Unicamp Central Animal Facilities and raised under specific pathogen-free condition. Male BALB/c mice, 8–10 weeks old, were matched for body weight before use. The animals were housed 8/cage and allowed free access to laboratory chaw and water. Animal experiments were done in accordance with institutional protocols and the guidelines of the Institutional Animal Care and Use Committee.

2.2. Tumour Model

Ehrlich's ascites tumour was maintained in BALB/c mice in the ascites form by serial transplantation. Tumour cell suspensions were prepared in balanced salt solution at pH 7.4 to final concentrations of 6×10^6 viable cells/ml. In all experimental protocols described, mice were inoculated intraperitoneally (i.p.) on day 0 with 6×10^6 viable tumour cells per mouse in a volume of 0.1 ml. Viability, assessed by the Trypan Blue exclusion method, was always found to be at least 95%.

2.3. Drug preparation and mice treatment

TITANOCENE X (Fig. 1) was prepared and purified by the group of Dr. Matthias Tacke (Conway Institute of Biomolecular and Biomedical Research, Chemistry Department, Centre for Synthesis and Chemical Biology, CSCB, University College Dublin, Belfield, Dublin 4, Ireland) starting from the appropriately substituted fulvene and titanium dichloride (Tacke et al., 2004a). The compound was diluted in DMSO (dimethylsulfoxide)/saline (1/10) immediately before use in appropriate concentrations. Groups of normal and EAT-bearing mice were treated for 3 consecutive days with different doses of the drug (2.5, 5.0, 10.0, 20.0 or 50.0 mg/kg) via the i.p. route, starting 24 h after tumour inoculation. After treatment, the animals were sacrificed for evaluation of natural killer cell activity and CFU-