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Antitumor properties of organometallic metallocene complexes of tin and germanium

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Summary. The antitumor activity of the four metallocene compounds decaphenylstannocene $[\eta^5\text{-}(C_6H_5)_5C_5]_2Sn(II)$, decabenzylstannocene $[\eta^5\text{-}(C_6H_5CH_2)_5C_5]_2Sn(II)$, decaphenylgermanocene $[\eta^5\text{-}(C_6H_5)_5C_5]_2Ge(II)$, and decabenzylgermanocene $[\eta^5\text{-}(C_6H_5CH_2)_5C_5]_2Ge(II)$, containing the main group IV elements tin or germanium as the central metal atom and two pentasubstituted cyclopentadienyl ring ligands in sandwich arrangement, were tested against Ehrlich ascites tumor in female CF1 mice. The complexes caused cure rates of 40% to 90% of the animals treated over rather broad dose ranges. With both germanocene complexes, no strong dose-activity relationship was manifest. The toxicity of all four metallocenes was low, the LD_{10} values of both stannocenes being 460 and 500 mg/kg, and those of both germanocenes higher than 700 mg/kg. Regarding the isolated pentasubstituted cyclopentadiene ligands $(C_6H_5)_5C_5H$ and $(C_6H_5CH_2)_5C_5H$, these also exhibited antitumor activity which was less pronounced than that of the metal-containing sandwich complexes. Decasubstituted stannocene and germanocene compounds represent a new type of non-platinum group metal antitumor agents structurally differing from known inorganic and organometallic cytostatics.

Introduction

Experimental studies during past decade have indicated antitumor activity for a variety of non-platinum group metal complexes (Köpf-Maier and Köpf 1987a). Organometallic compounds comprising early transition metals such as titanium or vanadium (Köpf and Köpf-Maier 1979; Köpf-Maier and Köpf 1979; Keller et al. 1982) as well as iron, copper, and gold complexes (Köpf-Maier et al. 1984; Elo and Lumme 1985; Mirabelli et al. 1985; Berners-Price et al. 1986), and organometallic compounds containing the main group elements tin and germanium (Crowe et al. 1980; Mulinos and Amin 1980; Kumano et al. 1978) were found to exhibit antiproliferative activity against various experimental, and in some cases, human tumors. Whereas the antitumor activity of the diorganotin complexes $R_2SnX_2L_2$ and related compounds is obviously limited to the experimental leukemia system P388 (Crowe et al. 1984), the germanium compounds germanium sesquioxide and spirogermanium are able to inhibit the growth of diverse fluid and solid tumors (Mulinos and Amin 1980; Kumano et al. 1978). In early clinical studies performed since 1979, spirogermanium has moreover shown some activity against advanced ovarian carcinomas and lymphocytic lymphoma (Weiss et al. 1984).

In the present study, new types of organometallic complexes of tin and germanium were investigated for antitumor properties against Ehrlich ascites tumor. The compounds tested were typical metallocene complexes in sandwich arrangement being composed by two substituted cyclopentadienyl rings and an interposed tin or germanium atom.

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Materials and methods

Substances

The metallocenes decaphenylstannocene $[\eta^5\text{-}(C_6H_5)_5C_5]_2Sn(II)$, decabenzylstannocene $[\eta^5\text{-}(C_6H_5CH_2)_5C_5]_2Sn(II)$, decaphenylgermanocene $[\eta^5\text{-}(C_6H_5)_5C_5]_2Ge(II)$, and decabenzylgermanocene $[\eta^5\text{-}(C_6H_5CH_2)_5C_5]_2Ge(II)$ were synthesized as described recently (Heeg et al. 1984; Schumann et al. 1985, 1986). They were the first air-stable, monomeric metallocene compounds containing germanium(II) or tin(II) as central metal atoms. As organic ligands they included two pentasubstituted cyclopentadienyl rings in sandwich arrangement, the ring planes being either arranged in a parallel position in the decaphenylmetallocenes (Fig. 1) or forming an angle of 31°–36 °C in the decabenzyl-substituted derivatives (Fig. 2). The compounds were characterized by infrared, Raman, nuclear

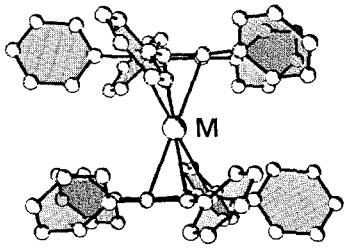


Fig. 1. Molecular structure of decaphenylmetallocenes (M = Sn, Ge)

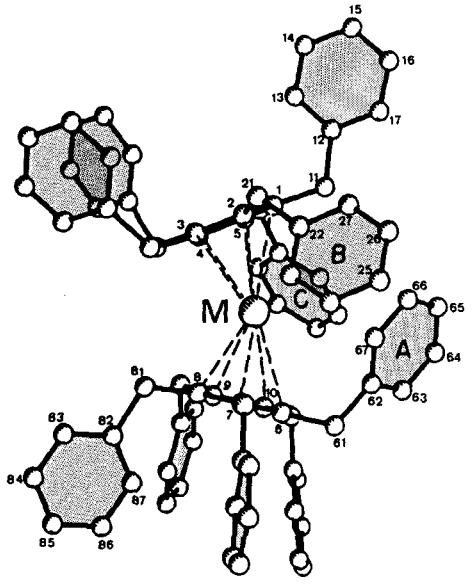


Fig. 2. Molecular structure of decabenzylmetallocenes (M = Sn, Ge)

magnetic resonance, mass spectroscopy, and X-ray structural analysis. No impurities were detected by these methods, and elemental analyses (C, H, Sn, or Ge) revealed deviations $\leqq 0.5\%$ of the calculated values. For comparison purposes, the isolated hydrocarbon ligands pentaphenylcyclopentadiene $(C_6H_5)_5C_5H$ and pentabenzylcyclopentadiene $(C_6H_5CH_2)_5C_5H$ were also synthesized and tested for tumor-inhibiting properties.

For antitumor testing, the decaphenylmetallocenes $[(C_6H_5)_5C_5]_2M$ with M = Sn or Ge were administered in doses from 20 to 600 mg/kg, rising by increments of 20 mg/kg, and the decabenzylmetallocenes $[(C_6H_5CH_2)_5C_5]_2M$ with M = Sn or Ge in doses ranging from 20 to 700 mg/kg. The pentasubstituted cyclopentadiene ligands were given in doses of 20 to 500 mg/kg with increments of 20 mg/kg. As the compounds were soluble in water to a limited extent only, they were dissolved in Tween 80 (Serva, Heidelberg) and then suspended in saline, the volume ratio Tween:saline always amounting to 1:9. The substance concentrations were so selected that each mouse received a total volume of 0.4–0.5 ml (0.02 ml/g body weight).

Animals

Female CF1 mice (Winkelmann, Paderborn) weighing 20–25 g were kept under standard conditions. They received food (Altomin) and tap water ad libitum.

Antitumor bioassay

The antitumor properties of the four metallocenes and both hydrocarbon ligands were tested against Ehrlich ascites tumor growing as an ascitic tumor in the peritoneal cavity of mice. Details of the experimental procedure have been described previously (Köpf-Maier et al. 1980, 1984). The compounds dissolved or suspended in the mixture of Tween and saline were administered as single injections 24 h after tumor transplantation. Every dose group consisted of 10 animals. Another 60 animals (6 groups of 10 mice) served as untreated, tumor-bearing control animals. They received 0.5 ml of the Tween-saline mixture 1/9, v/v) without drug additon.

The number of deaths was registered daily. Deaths within 7 days after substance administration were related to substance toxicity, those occurring later defined as tumor deaths. All animals dying later than day 8 after tumor transplantation showed macroscopic signs of tumor disease. On day 90 after tumor transplantation, the survival rate was determined, and animals with no recognizable signs of tumor development were considered as cured.

Results

All control animals died due to tumor development between day 17 and 23 after tumor transplantation, the mean value of survival time amounting to 20.2 ± 2.1 days.

Treatment with stannocenes

Both stannocenes tested in the present study were characterized by antiproliferative activity against Ehrlich ascites tumor, and animals survived when doses higher than 100 mg/kg were used (Figs. 3 and 4).

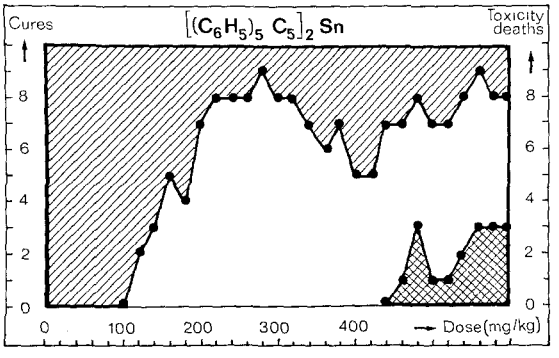


Fig. 3. Dose-activity (left graph) and dose-lethality (right graph) relationships of decaphenylstannocene. ▨ Tumor deaths; ▩ deaths due to substance toxicity; ☐ surviving, cured animals

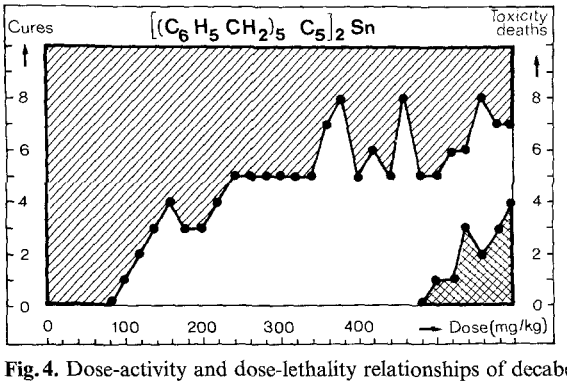


Fig. 4. Dose-activity and dose-lethality relationships of decabenzylstannocene. For further explanations see legend to Fig. 3

In the dose ranges of 140–160 mg/kg (decaphenylstannocene) or 220–500 mg/kg (decabenzylstannocene) 40%–90% of the animals treated were cured of tumor, whereby decaphenylstannocene was obviously more effective inducing a higher mean cure rate of 68% and an optimum cure rate of 90% in comparison to 55% and 80%, respectively, for decabenzylstannocene. As lethal dose $(LD)_{10}$ values, doses of 460 and 500 mg/kg were found in the case of decaphenylstannocene and decabenzylstannocene. The LD_{50} and LD_{100} values of both compounds were greater than 600 mg/kg.

Treatment with germanocenes

Both metallocenes containing germanium as the central metal atom effected cure rates of 40%–90% over a broad dose range of more than 400 mg/kg (Figs. 5 and 6). There were pronounced variations between the cure rates of neighboring dose levels resulting in agitated courses of the graphs representing the dose-activity relationships of decaphenylgermanocene and decabenzylgermanocene (Figs. 5 and 6). The mean cure rate of both germanocenes was 60% in the dose ranges 280–700 and 300–700 mg/kg. Toxic deaths did not occur within the experimental dose ranges up to 700 mg/kg. Nevertheless, higher doses were not used because it was very difficult to dissolve or suspend them sufficiently in the injection volume of 0.5 ml.

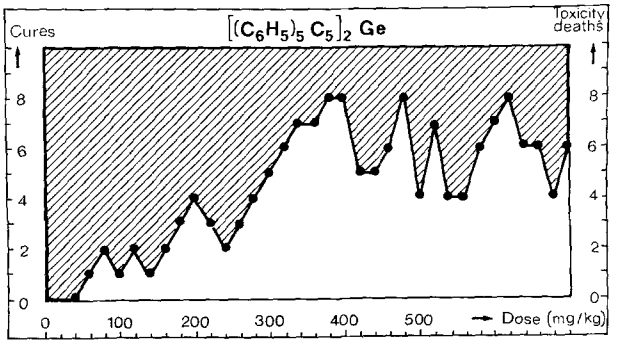


Fig. 5. Dose-activity relationship of decaphenylgermanocene. For further explanations see legend to Fig. 3

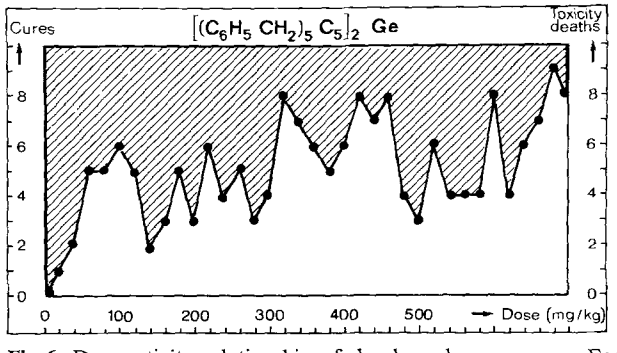


Fig. 6. Dose-activity relationship of decabenzylgermanocene. For further explanations see legend to Fig. 3

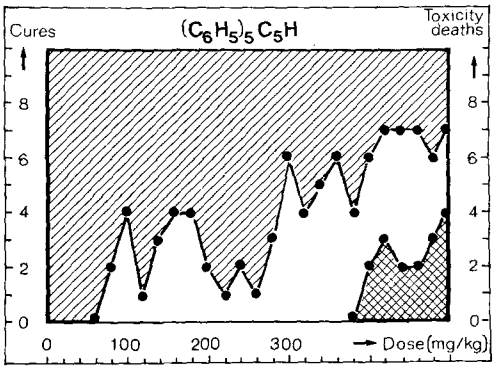


Fig. 7. Dose-activity and dose-lethality relationships of pentaphenylcyclopentadiene. For further explanations see legend to Fig. 3

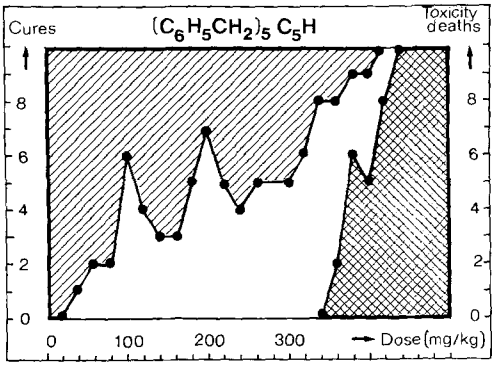


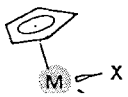
Fig. 8. Dose-activity and dose-lethality relationships of pentabenzylcyclopentadiene. For further explanations see legend to Fig. 3.

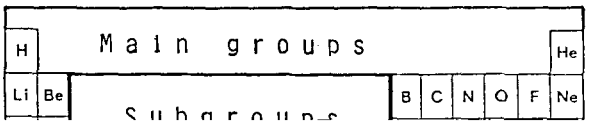
Treatment with pentasubstituted cyclopentadienes

The isolated hydrocarbon ligands pentaphenylcyclopentadiene and pentabenzylcyclopentadiene also showed antitumor activity against Ehrlich ascites tumor (Figs. 7 and 8), whereby maximum cure rates of 60 and 80% were induced. In comparison to the metal-containing complexes, the ranges of doses inducing cure rates exceeding 40% were comparably narrow and extended from 300 to 380 mg/kg in the case of pentaphenylcyclopentadiene and 180 to 340 mg/kg for pentabenzylcyclopentadiene. Within these dose ranges, the mean cure rates were 50% and 55%. Toxic deaths were caused by both compounds used at doses higher than 380 or 340 mg/kg, respectively, the LD_{50} and LD_{100} values of pentabenzylcyclopentadiene being 390 and 440 mg/kg.

Discussion

The stannocene and germanocene complexes investigated in the present study are the first main group metallocenes for which antitumor activity was shown. They represent a new type of non-platinum group metal antitumor agent, the main exponents of which





$Ga^{3+}(NO_3^-)_3$

(G)

considered. Regarding the molar concentrations leading to equivalent therapeutic and toxic effects (Table 1), about 2-fold higher molar doses of the hydrocarbon ligands are generally necessary to cause corresponding effects, which would be in accordance with the presence of two active cyclopentadienyl ring ligands per metallocene molecule. Thus, the pentasubstituted cyclopentadienyl ring ligands may essentially contribute to the antitumor activity of the decasubstituted stannocene and germanocene complexes tested in the present study.

The decasubstituted stannocene and germanocene complexes differ from unsubstituted titanocene and vanadocene compounds where the isolated organic ligands cyclopentadiene and dicyclopentadiene certainly exhibit local tumor-inhibiting effects, but only in 10- to 20-fold higher molar doses than the metal-complexed compounds, and moreover lack systemic antiproliferative and antiarthritic activities (Köpf-Maier and Köpf 1987b; Fairlie et al. 1987) which are characteristic for titanocene and vanadocene dichloride.

As the stannocene and germanocene complexes described in the present study are only poorly soluble in water to a lesser extent than other organometallic antitumor agents, their biological administration is problematic and limited by this property. Future investigations are necessary to see if the water solubility of stannocene and germanocene complexes can be improved by chemical modification of the molecules and if, perhaps, the antitumor properties can be intensified by this procedure.

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