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Tumor Inhibition by Metallocenes: Activity against Leukemias and Detection of the Systemic Effect*

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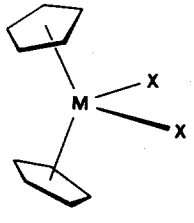
Abstract—*The antitumor activity of two representatives of the metallocene dihalides, titanocene dichloride (TDC) and vanadocene dichloride (VDC), is investigated by the use of various experimental tumor systems. Against the lymphoid leukemia* L1210 *and the lymphocytic leukemia* P388, *TDC and VDC induce significant increases of life span corresponding to T/C values which exceed* 125%. *On treating advanced stages of fluid EAT with single doses, TDC causes, when applied within* 5 days *after transplantation, the survival of* 80–100% *of the treated animals, whereas VDC cures progressed stages of fluid EAT to a much minor extent. Investigations using a solid EAT subcutaneously growing in the nuchal region show that the intraperitoneally applied metallocene dihalides are able to inhibit tumor growth in a highly significant manner so that the minimum tumor sizes attain* 14% *(TDC) or* 31% *(VDC) of the control values. This result indicates that the antiproliferative activity of the metallocene dihalides is based on a systemic effect.*

INTRODUCTION

THE METALLOCENE dihalides $(C_5H_5)_2MX_2$ represent the first example of a group of organometallic compounds which exhibit cancerostatic properties by their own [1–3].



Interestingly, organotin complexes $R_2SnX_2 \cdot L_2$ which possess a structural relationship to the metallocene dihalides by an analogous *cis*-dihalo-metal(IV) moiety also reveal tumor-inhibiting properties [4].

In previous studies the structure–activity relation of the metallocene dihalides was investigated against the fluid Ehrlich ascites tumor (EAT) system. The following results could be pointed out: (i) The antitumor activity of the metallocene dichlorides $(C_5H_5)_2MCl_2$ is strongly dependent on the metal central atoms M [2, 3, 5, 6]; (ii) All titanocene dihalides $(C_5H_5)_2TiX_2$ with X = F, Cl, Br, I, NCS, N_3 show similarly extensive tumor-inhibiting properties [7]; (iii) Chemical modification of the cyclopentadienyl rings of $(C_5H_5)_2TiCl_2$ diminishes the cancerostatic effectiveness in dependence upon the degree of the modification [8].

In the present work the antitumor properties of titanocene dichloride (TDC) and vanadocene dichloride (VDC), two representatives of the metallocene dihalides with X = Cl and M = Ti or V, respectively, are investigated against other experimental tumor systems, i.e., the lymphoid leukemia L1210 and the lymphocytic leukemia P388 as well as against a subcutaneously growing, solid EAT.

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MATERIALS AND METHODS

Experimental tumors

Lymphoid leukemia L1210 *and lymphocytic leukemia* P388. The tumors were provided by

the NCI Liaison Office, Institut Jules Bordet (Bruxelles, Belgium). The tumor propagation and the transplantation for testing were performed according to the NCI protocols [9]. Female DBA/2 mice (Zentralinstitut für Versuchstierzucht, Hannover, Germany) were used for propagation; the testing group for each tumor consisted of 180 female BDF_1 mice (Gl. Bomholtgård, Ry, Denmark).

Fluid Ehrlich ascites tumor. The fluid EAT was transferred for propagation and for testing as described [7].

Solid Ehrlich ascites tumor. To produce solidly growing EAT, the ascites of donor mice bearing the fluid EAT for 8 days was harvested from the peritoneal cavity and diluted with saline 1:7 (v:v). Volumes containing about 10^7 cells were subcutaneously inoculated in the nuchal region of each of the 240 experimental animals (CFI mice; F. Winkelmann, Paderborn, Germany).

Substances and application

Titanocene dichloride (TDC) and vanadocene dichloride (VDC) were prepared and purified according to methods described in the literature [10]. Elemental analyses were within $\pm 0.5\%$ of the theoretical values. *cis*-Diamminedichloroplatinum(II) (DDP) (Ventron, Karlsruhe, Germany) was used as positive control compound.

L1210 *and* P388. Doses of 10, 20, 30, ..., 130 mg/kg TDC or VDC were applied intraperitoneally (i.p.) and administered in 0.5 ml of a DMSO–saline mixture (1:9 = v:v) as single doses on day 1 after tumor transplantation (p.t.t.). Each dose group consisted of six animals. For L1210 as well as for P388, 24 animals each served as untreated tumor-bearing controls. They received on day 1 p.t.t. 0.5 ml of the DMSO–saline solution without drug addition.

Fluid EAT. Fluid EAT was treated at seven different stages of progression with one of the three substances DDP (10 mg/kg), TDC (70 mg/kg), or VDC (80 mg/kg) dissolved in 0.5 ml DMSO–saline mixture. Therefore 21 groups of 10 animals each were formed. The animals in each group were given single i.p. injections on one of days 1, 2, 3, 4, 5, 6 or 7 p.t.t. Twenty additional animals were used as untreated controls. They received 0.5 ml DMSO–saline solution on day 1 p.t.t.

Solid EAT. The animals bearing a solid EAT in the nuchal region obtained 2-fold i.p. injections (2×10, 2×20, ..., 2×60 mg/kg) on day 1 and 3 p.t.t. in the case of TDC, and 3-fold i.p. injections (3×10, 3×20, . . . , 3×60 mg/kg) on day 1, 3 and 5 p.t.t. in the case of VDC. The positive control compound DDP was administered twice on day 1 and 3 in single doses of 10 mg/kg. Each dose group consisted of 15 CF1 mice. Another 30 animals received i.p. a DMSO–saline mixture on days 1, 3 and 5 and served as negative control animals.

Evaluation of the experiments

L1210 *and* P388. The deaths were recorded daily. T/C values were computed from the percentage mean survival time of each dose group related to the controls. By subtraction of 100%, the values of the increase in life span (ILS) given in Figs. 1 and 2 were

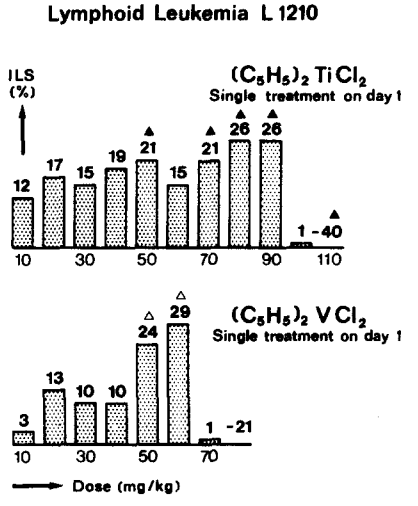

Fig. 1. Dose-dependent increase in life span (ILS) after treatment of L1210 with TDC or VDC on day 1 p.t.t. △ Significant ($2P < 0.05$); ▲ highly significant ($2P < 0.01$) difference between survival time of experimental and control animals.

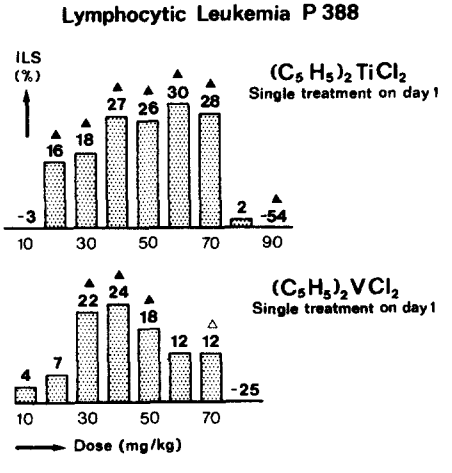

Fig. 2. Dose-dependent increase in life span (ILS) after treatment of P388 with TDC or VDC on day 1 p.t.t. △ Significant ($2P < 0.05$); ▲ highly significant ($2P < 0.01$) difference between survival time of experimental and control animals.

obtained. Additionally, the statistical significance was examined by the use of the Wilcoxon–Mann–Whitney *U*-test.

Fluid EAT. After daily weighing the animals and noting the number of deaths, the survival rate for each group was determined on day 120 p.t.t. (Fig. 3).

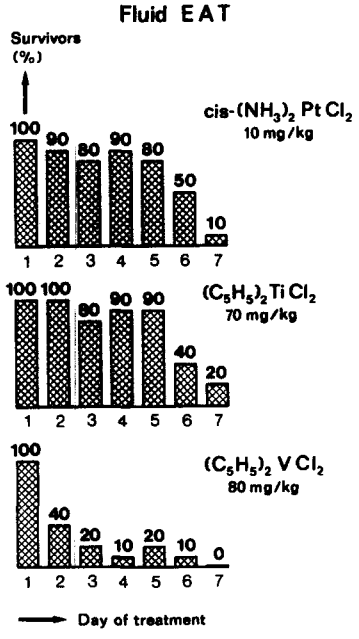

Fig. 3. Survival rate after treatment of fluid EAT at various stages after transplantation (day 1–7 p.t.t.) with single doses of DDP, TDC or VDC.

Solid EAT. On day 8 p.t.t. all animals were killed. The solid tumors were totally resected and weighed with an accuracy of ± 1 mg. The tumor weights as well as the T/C values, calculated by comparing the mean tumor weights of each dose group to the mean tumor weight of the controls are arranged in Figs. 4

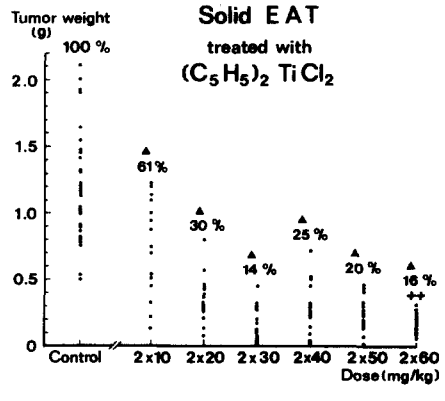

Fig. 4. Ranged weights of solid EAT on day 8 p.t.t. after i.p. treatment with TDC. The numbers on top represent T/C values. △ Significant (2P < 0.05), ▲ highly significant (2P < 0.01) difference of the tumor weights compared to the controls. + Toxic death before day 8 p.t.t.

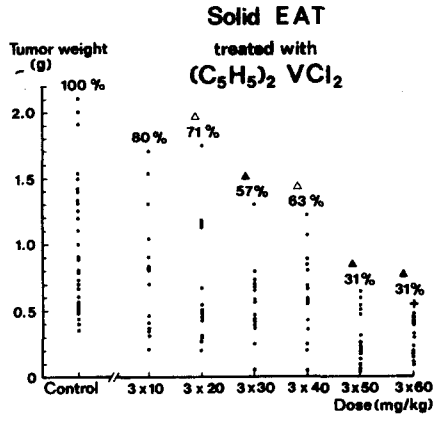

Fig 5. Ranged weights of solid EAT on day 8 p.t.t. after i.p. treatment with VDC. For symbols see legend to Fig. 4.

and 5. Statistical evaluation was made by the Wilcoxon–Mann–Whitney *U*-test.

RESULTS

L1210 *and* P388 (*Figs. 1 and 2*)

Whereas the control animals survived 6–9 (mean value 7.45) days in the case of the lymphoid leukemia L1210 and 8–12 (10.04) days in the case of the lymphocytic leukemia P388, the mean survival time was prolonged after treatment with TDC and VDC in dependence upon the applied doses. In both tumor systems, TDC caused highly significant ILS values over a relatively wide dose range, mostly corresponding to T/C values higher than the limit of 125% [9]. The decline of mean survival time after application of higher doses than 100 or 80 mg/kg, respectively, is due to an increasing number of toxic deaths. VDC also delayed the animals' deaths; however, the optimum dose range was more narrow than for TDC. Whereas the maximum T/C value attained 129% when L1210-bearing mice were treated with VDC, the T/C values only increased to 124% in the case of P388.

Fluid EAT (*Fig. 3*)

These experiments were performed to examine the therapeutic activity of TDC and VDC in comparison to DDP against progressed stages of EAT. Whereas all untreated control animals died within 10–17 (mean value 15.45) days p.t.t., both DDP and TDC evoked 90–100% cures when applied in optimum doses on day 1 and 2 p.t.t. When administered on day 3, 4 or 5, these compounds still caused the survival of 80–90% of the treated animals. Thereafter, the surviving rate decreased rapidly. In contrast to DDP

and TDC, the curing potency of VDC was much less when advanced stages (beginning with day 2) of EAT were treated.

Solid EAT (*Figs.* 4 *and* 5)

The proliferation of the EAT growing subcutaneously in the nuchal region was strongly and dose-dependently inhibited by multiple i.p. treatment with TDC or VDC. After application of TDC at the lowest dose level (2 × 10 mg/kg), the tumor sizes decreased to 61% of the controls. Values of 14–25% were observed after application of higher doses of TDC, and the highest dose caused two toxic deaths. Compared to DDP which evoked a T/C value of 19% at 2 × 10 mg/kg (the absolute tumor weights ranging from 0.03–0.46 g), TDC was apparently able to inhibit the growth of solid EAT to a similar extent. VDC, on the other hand, also reduced the tumor sizes markedly. However, the active doses were higher than for TDC and the minimum T/C value of 31% (after application of 3 × 50 mg/kg) was near the dose of 3 × 60 mg/kg which produced the first toxic death.

DISCUSSION

Several metallocene dihalides are characterized by strong tumor-inhibiting properties against EAT when applied as usual on day 1 p.t.t. [1–3, 5–7]. Whereas TDC and VDC, as well as the merely inorganic complex DDP, induce 100% cures at this time, only TDC and DDP show up to day 5 marked tumor-inhibiting potencies against advanced stages of fluid EAT. Using the leukemias L1210 and P388 as alternative tumor systems, VDC exhibits even on day 1 a weakened therapeutic influence compared to TDC. These results are surprising because VDC suppresses cellular growth of EAT *in vitro* in a 100-fold lower concentration (5×10^{-6} mol/l) than does TDC (5×10^{-4} mol/l) [11].

All these antiproliferative effects of TDC and VDC *in vivo* as well as *in vitro* might have been caused by a local effect of the substances. Therefore, we used a subcutaneously growing solid EAT for which the sensitivity to metallocene dihalides is known in the fluid form [1–3, 5–7], and treated animals bearing this tumor in the nuchal region by multiple injections of TDC and VDC into the peritoneal cavity. The result of this experiment showing that both TDC and VDC strongly inhibit the tumor growth over a wide dose range indicates that the metallocene dihalides are capable of inhibiting tumor proliferation by a systemic effect. Interestingly, the effectiveness of VDC is again less extensive than that of TDC and DDP. The latter two compounds, however, show very similar growth inhibiting potencies leading to a size reduction of 80–90% related to the controls.

In conclusion, the metallocene dihalides represent a group of organometallic compounds which show antitumor activity, based on a systemic effect, against various experimental tumor systems. It therefore seems promising to perform further studies with this new class of antitumor agents.

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