Antitumor and Toxicologic Properties of the Organometallic Anticancer Agent Vanadocene Dichloride

M. SATYA MURTHY*, LEELA N. RAO
Evanston Hospital, 2650 Ridge Avenue Evanston, Ill. 60201, U.S.A.
LOUIS Y. KUO, JEFFREY H. TONEY and TOBIN J. MARKS*
Department of Chemistry, Northwestern University, Evanston, Ill. 60201, U.S.A.

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Abstract

We report here on the antineoplastic, toxicologic, and transmembrane transfer properties of vanadocene dichloride (VDC)**, a representative metallocene dihalide. VDC is cytotoxic to HEp-2 human epidermoid carcinoma cells, *in vitro*, in a dose dependent manner, with a D_o value (dose increment reducing the survival fraction by 1/e) of 0.530 ± 0.005 μg/ml. Under similar experimental conditions, the D_o for cisplatin (CDDP) against these cells is 0.46 ± 0.08 μg/ml. In a murine mammary adenocarcinoma (TA3Ha) system, 125 μg/ml VDC inhibits the tumor-forming ability of 10^5 cells upon i.p. inoculation into syngeneic Strain A mice. The transmembrane transfer rate constants for the metal uptake of VDC and CDDP by TA3Ha cells *in vitro* were found to be $3.3 \pm 0.8 \times 10^{-4}$ min^{-1} and $12 \pm 2.0 \times 10^{-4}$ min^{-1}, respectively. *In vivo* studies with TA3Ha cells show that two i.p. treatments of 20, 40, and 60 mg/kg VDC increase the host survival by 30, 50, and 90%, respectively. Under similar conditions, 2, 4, and 6 mg/kg CDDP (equitoxic dose levels) prolong the host survival by 50, 75, and 83%, respectively. Morphological, blood urea nitrogen level, and serum creatinine level data for Strain A mice treated with 60 mg/kg VDC give no evidence of renal or small intestinal damage. However, changes in the liver consistent with fatty cell degeneration are observed in these mice.

Introduction

We report here chemical and biological investigations relevant to the antineoplastic efficacy of vanadocene dichloride (VDC, $(C_5H_5)_2VCl_2$), a representative metallocene dihalide. Our studies were prompted by the reports of Köpf-Maier *et al.* that some $(C_5H_5)_2MX_2$ complexes (M = Ti, V, Mo, Nb; X = F, Cl, Br, I, NCS, N_3) exhibit antineoplastic activities against a wide spectrum of experimental murine tumors [1–3], and in the case of $(C_5H_5)_2TiX_2$ (X = Cl, Br), against human tumors xenografted into athymic nude [4] mice. It has also been demonstrated that the respective metals of several $(C_5H_5)_2MX_2$ systems accumulate in the nuclear heterochromatin of tumor cells [5] and inhibit DNA synthetic [6, 7] and mitotic activities [1]. These results and molecular structural similarities to CDDP [8–11] have led to speculation that the carcinostatic activities of the two classes of compounds may reflect similar DNA-binding mechanisms [1].

Our studies of $(C_5H_5)_2TiCl_2$ and VDC aqueous chemistries have revealed marked dissimilarities to that of CDDP [12]. Thus, hydrolytic replacement of chloride by H_2O in the $(C_5H_5)_2MCl_2$ compounds is far more extensive and rapid than for CDDP. With regard to ancillary ligands, VDC exhibits significant hydrolytic stability of the M–C_5H_5 bonds while $(C_5H_5)_2TiCl_2$ does not. In contrast to CDDP, VDC exhibits labile, predominantly phosphate-centered binding to DNA constituents, with little disruption of Watson–Crick base-pairing [13]. We have also found that vanadium from i.p. administered VDC is cleared more rapidly from various organs of Strain A mice than is CDDP [14].

The hydrolytic stability of the $(C_5H_5)_2V^{2+}$ framework clearly renders VDC an ideal metallocene dihalide candidate for detailed antitumor and toxicologic studies. In the present contribution [15], we focus upon the cytotoxicity against a human epidermoid carcinoma-derived cell line and upon a transplantable mouse mammary adenocarcinoma. In addition, we discuss the *in vivo* toxicity and drug-related kidney, liver, and small intestine damage in Strain A mice, as well as *in vitro* rates of VDC/CDDP uptake by the above murine tumor cells. Some of

*Authors to whom correspondence should be addressed.
**The abbreviations used are: VDC, vanadocene dichloride, $(C_5H_5)_2VCl_2$; CDDP, *cis*-dichlorodiammineplatinum(II), *cis*-$Pt(NH_3)_2Cl_2$; BUN, blood urea nitrogen; C_e, extracellular concentration, C_i, intracellular concentration; HBSS, Hanks' balanced salt solution; ILS, increase in life span; DMSO, dimethyl sulfoxide; DMF, dimethylformamide.

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these parameters are presented in comparison with those of CDDP, a highly effective transition metal-based anticancer drug.

Experimental

Materials and Methods

VDC was purchased from Strem Chemical Company (Newburyport, Mass.) and purified by anaerobic Soxhlet extraction with CH_2Cl_2 as described earlier [12]. Purity was checked by infrared, electron spin resonance, and proton nuclear magnetic resonance spectroscopies as well as by elemental analysis. The purified compound was handled under prepurified nitrogen using standard Schlenk techniques and was stored under nitrogen in sealed vials. All solvents were appropriately purified and degassed as has been described earlier [12]. Injectable Platinol (CDDP) was purchased from Bristol Laboratories, Syracuse, N.Y., and used without further purification. Nutrient medium F-10, HBSS, trypsin, and fetal bovine serum were purchased from the Grand Island Biological Company (Grand Island, N.Y.). Ficoll-paque was purchased from Pharmacia Chemicals (Piscataway, N.J.). Transplantable mouse mammary adenocarcinoma line TA3Ha [16] (syngeneic to Strain A mice) and human epidermoid carcinoma HEp-2 cells were used. Maintenance, preparation, and the biological properties of these cells have been described earlier [17, 18].

Strain A female mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. They were 6–8 weeks of age and weighed an average of 20 g at the beginning of each experiment.

Cytotoxicity of VDC and CDDP against HEp-2 Cells

This was assessed by an *in vitro* colony formation assay as has been described earlier [19]. VDC was dissolved in DMSO (in which it is stable for periods of days) immediately prior to use and diluted to appropriate concentrations in HBSS (highest concentration of DMSO = 0.2%). CDDP was dissolved in HBSS immediately prior to use.

HEp-2 cells were harvested by trypsinization and a monodispersed cell suspension prepared in F-10 medium supplemented with 20% fetal bovine serum. These cells were seeded into T25 flasks at a density of 1000 cells/flask/5 ml growth medium. About 18 h later, the cells were exposed to various concentrations of VDC or CDDP for 30 min at 37 °C. Controls were similarly exposed to the solvent without the drug. At the end of the treatment period, the cells were washed in HBSS and fed with fresh drug-free medium. Colonies (closely packed group of 50 or more cells) were enumerated after an incubation period of 8 to 10 days. The survival fraction, expressed as per cent of untreated controls (plating efficiency 20–30%), was plotted as a function of drug dose. The D_o values (the dose increment that reduced the survival fraction by a factor of 1/e on the exponential portion of the curve) from at least 3 independent experiments, each with triplicate plates per dose, were obtained as described earlier [19].

Cytotoxicity of VDC against TA3Ha Cells

This was assessed by tumor transplantation assay as has been described earlier [18, 19]. Freshly harvested TA3Ha cells were washed in HBSS and treated with various concentrations of VDC for 60 min at 37 °C. The controls were similarly treated with the solvent containing no VDC. At the end of the treatment, cells were washed in drug-free medium and injected i.p. into Strain A mice at a dose of 100 000 cells/mouse. The mice were observed for 60 days for tumor-induced host death. The mean survival periods of mice, in days, were plotted as a function of the drug dose using a linear least-squares fitting procedure.

Toxicity Studies

VDC, diluted to appropriate concentrations in 10% DMSO–saline, was injected i.p. in 1 ml volumes into Strain A mice. The mice were observed for 15 days for mortality. Morphological damage to the kidney was quantitated by evaluating the tubular necrosis, karyorrhexis, and pyknosis of cells [20, 21]. At least 600 tubules around the subcortical glomeruli were examined. The intestinal damage was quantitated by calculating the surface to volume ratio of the jejunal villi as has been described earlier [21, 22]. Histological damage, if any, in the liver tissue was qualitatively examined.

Kinetic Quantitative Determination of Blood Urea Nitrogen and Creatinine

Mice were injected i.p. with 60 mg/kg VDC (therapeutic dose used) and groups of 3 mice were sacrificed by exposure to ether on days 1, 3, 5, 7, and 12. Blood from these and a group of 10 untreated control mice was collected from inferior vena cava. BUN levels were determined using Worthington Statzyme BUN16 Reagents (Cooper Biomedical, Freehold, N.J.) and a Gilford 4 computer-assisted spectrophotometer. The test conditions were: temperature = 30 °C, wavelength = 340 nm, serum volume = 10 µl, reagent volume = 1 ml, $T1$ (incubation time) = 90 s, and $T2$ (reaction time) = 15 s. Creatinine levels were determined using the Worthington Creatinine Reagent Set (Cooper Biomedical, Freehold, N.J.) and a Gilford 4 computer-assisted spectrophotometer. The test conditions were: temperature = 30 °C, wavelength = 510 nm, serum volume = 50 µl, reagent volume = 1 ml, $T1$ = 15 s, $T2$ = 15 s.

Antitumor Effects of VDC and CDDP

These were assessed using the TA3Ha ascites tumor model [16]. Freshly harvested TA3Ha cells (10^4) were injected i.p. into Strain A mice. On days 2 and 7 after inoculating the tumor cells, the mice were injected i.p. with various doses of freshly dissolved VDC or CDDP in 1 ml volume. Controls received 1 ml of solvent alone. Mice were examined daily to assess the host deaths. Per cent increase in life span (%ILS) as a function of drug dose was determined.

Uptake of VDC and CDDP by TA3Ha Cells

Freshly harvested TA3Ha cells were washed in HBSS and dispensed into preweighed tubes at a density of 100 X 10^6 cells/2ml/tube. Known amounts of CDDP or VDC were added to the cell suspension and incubated at 37 °C for 10 to 60 min. After appropriate periods of incubation, the cells were sedimented and washed rapidly with 0.9% (*w*/*v*) NaCl solution. The supernatants from each sample were pooled for determining the extracellular amount (C_e) of platinum or vanadium. The cell pellets were dried in an oven at 70 °C overnight and the dry weights determined. The dried pellets were next digested in 1 or 2 ml of concentrated HNO_3. These cell digests were used for determining the intracellular/cell-bound (C_i) amount of platinum or vanadium. The quantitation of metal in the extracellular and cell-bound fractions was carried out by flameless atomic absorption spectroscopy as has been described by us previously [12]. The cell-bound metal per unit weight (dry) of cells was obtained. C_i/C_e was then plotted as a function of time (t).

Results

Cytotoxicity of Vanadocene Dichloride

The effect of VDC on the colony-forming ability of HEp-2 human tumor cells *in vitro* is presented in Fig. 1. The cell survival curve for VDC is characterized by D_o of 0.53 ± 0.005 μg/ml and a negligible shoulder (D_q). The cell survival curve for CDDP is very similar, showing a D_o of 0.46 ± 0.08 μg/ml. These results indicate that VDC is 87% as cytotoxic as CDDP against human HEp-2 epidermoid carcinoma cells under the present identical experimental conditions.

The cytotoxicity of VDC against TA3Ha mouse mammary adenocarcinoma cells as determined by the tumor-forming ability in Strain A mice is presented in Fig. 2. The cells treated with up to 32 μg/ml VDC form tumors in 100% of the injected mice. The host survival period and the dose of VDC are linearly related with a slope of 0.22 and correlation coefficient of 0.99. Tumor formation by the cells treated with 64 and 125 μg/ml VDC is 69% (18/26) and 0% (0/10), respectively.

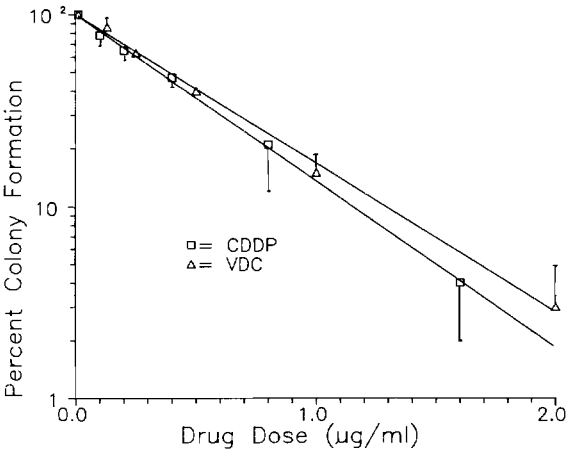

Fig. 1. Effect of VDC and CDDP on the colony formation by HEP-2 cells. Eighteen hour old HEp-2 cultures were exposed to varying concentrations of VDC (△) and CDDP (□) for 30 min at 37 °C and allowed to form colonies for 8–10 days. Colony formation, expressed as percentage of controls, is plotted as a function of drug dose (μg/ml). The plating efficiency of the untreated controls was 20–30%. Half of each error (s.d.) bar has been truncated for legibility.

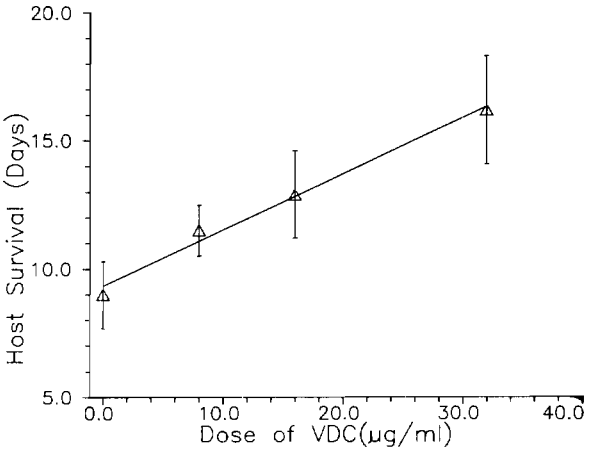

Fig. 2. Cytotoxicity of vanadocene dichloride (VDC) against TA3Ha mouse mammary adenocarcinoma as assayed by tumor formation (mean survival periods) in Strain A mice. Bars, s.d.

Toxicologic Studies

A single i.p. injection of VDC, at up to 60/mg/kg, causes no mortality in Strain A mice nor any detectable body weight loss. LD10, LD50, and LD100 values are 70, 80, and 100 mg/kg, respectively (Table I). Lethality is linearly related to the dose of VDC administered, ($r = 0.99$). Up to 140 mg/kg VDC administered in divided doses (20 mg/kg/day for 7 days) causes no toxicity-related deaths. VDC-induced toxic deaths occur within 24 to 48 h of drug administration, and surviving animals showed no body weight loss.

TABLE I. Lethality, Kidney and Intestinal Damage of VDC in Nontumor Bearing Strain A Mice

VDC Dose (mg/kg)	Mortality	% Abnormal proximal renal tubules	Intestinal damage s/v of jejunal villi, mean ± s.d.
60	0/30	4 ± 0.4	27 ± 1.6
70	1/10 (10)[a]	ND[c]	ND
80	5/10 (50)[a]	8 ± 1.6	26 ± 4.0
90	8/10 (80)[a]	ND	ND
100	8/8 (100)[a]	ND	ND
Control	NA[b]	4 ± 0.2	30 ± 0.3

Strain A mice were subjected to a single i.p. injection schedule of a vanadocene dichloride (VDC) solution. [a]Numbers in parentheses are percentage of mortality. [b]NA = not applicable. [c]ND = not done.

Since our previous VDC biodistribution studies [14] indicated that the principal organs of mice that accumulate vanadium are the kidney, liver, and small intestine, we focused our attention on examining these organs for toxicity related alterations.

Histological architecture of the kidney (Fig. 3), as well as an assessment of the per cent abnormal proximal renal tubules in mice on day 5 of treatment with 60 mg/kg VDC (therapeutic dose) are similar to those in the untreated control mice (Table I). The serum levels of blood urea nitrogen and creatinine in these mice on day 5 of treatment (Table II) give no evidence of functional alterations in this organ. The small intestinal tissues of VDC-treated mice show no discernible abnormality (Fig. 4).

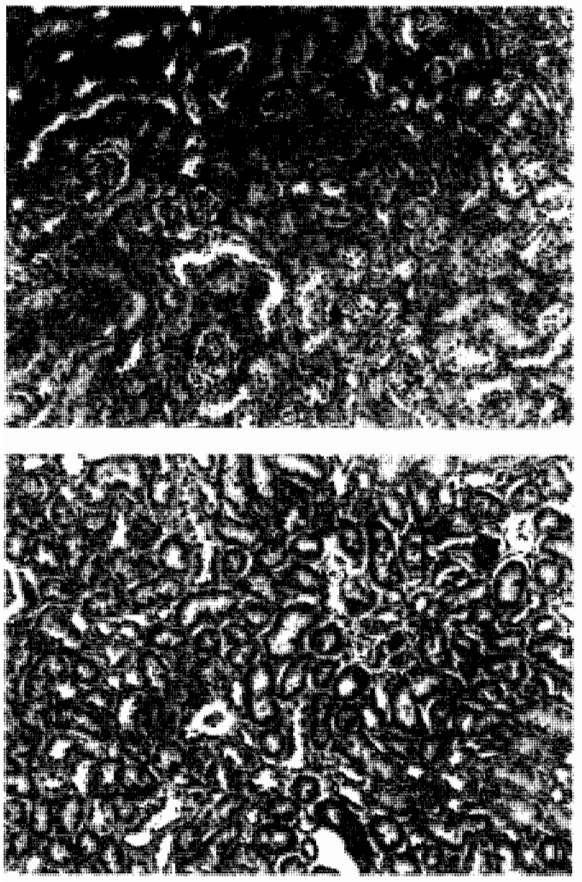

Fig. 3. (a) Histological section of normal mouse kidney showing the subcortical glomeruli and tubules. ×96. (b) Histological section of mouse kidney 5 days after treatment with mg/kg VDC. ×96.

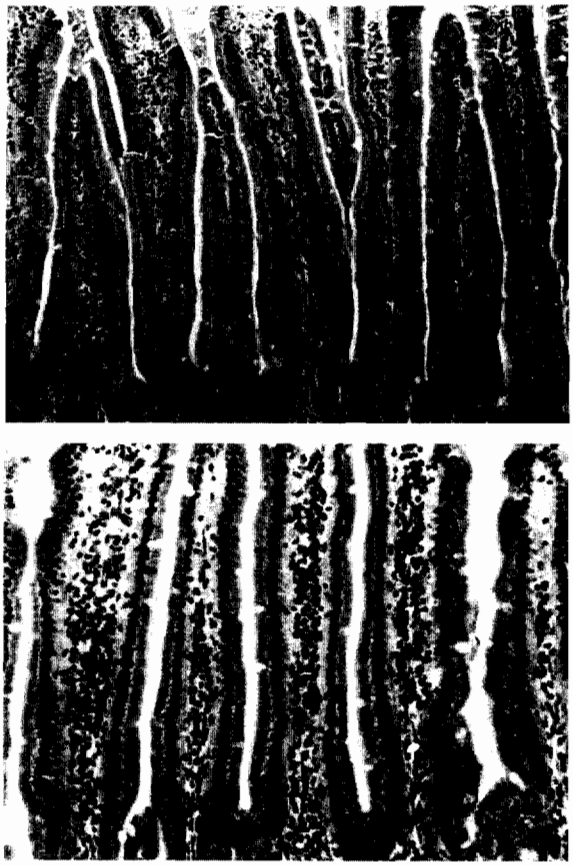

Fig. 4. (a) Histological section of jejunal mucosa from an untreated mouse showing normal villi and crypts. ×96. (b) Histological section of jejunal mucosa day 5 after treatment with 60 mg/kg VDC. ×96.

TABLE II. Nephrotoxicity of VDC

Days after treatment	n^a	BUN (mg/dl) mean ± s.d.	Creatinine (mg/ml) mean ± s.d.
Control	10	22.3 ± 1.71	0.61 ± 0.11
Day 1	5	11.9 ± 1.50	0.56 ± 0.10
Day 3	5	15.1 ± 1.97	0.58 ± 0.11
Day 5	5	18.6 ± 2.68	0.76 ± 0.08
Day 7	5	20.0 ± 2.32	0.61 ± 0.04
Day 12	5	26.1 ± 5.47	0.57 ± 0.06

Strain A mice were subjected to a single i.p. injection schedule of a vanadocene dichloride (VDC) solution (60 mg/kg) and were sacrificed on days 1, 3, 5, 7, and 12 for blood urea nitrogen (BUN) level and serum creatinine level determinations. [a] n = number of mice.

The quantitative analysis of the jejunal villous atrophy in the mice treated with 60 mg/kg VDC also shows no evidence of intestinal toxicity (Table I). Histological examination of the liver of mice treated with 60 mg/kg VDC indicates fatty cell degeneration, as evidenced by the presence of intracytoplasmic vacuoles (lipid droplets) (Fig. 5). That these vacuoles indeed were lipid droplets was confirmed histochemically by staining the frozen tissue sections with Sudan IV stain. Ultrastructural changes in the liver as well as the levels of serum enzymes and bilirubin are currently under investigation and will be presented in a separate communication.

Antitumor Effects of VDC and CDDP

Using nonlethal doses of VDC and CDDP, a comparative study of their antitumor effects was conducted in the TA3Ha ascites tumor model. Mice injected with 10000 cells were treated with varying doses of VDC or CDDP on days 2 and 7 of tumor inoculation. The results of this study (Fig. 6) show that the per cent increase in life span (%ILS) of mice treated with 20, 40, and 60 mg/kg VDC was 30, 50, and 90, respectively. The %ILS of mice treated with 2, 4, and 6 mg/kg CDDP was 50, 75, and 83, respectively.

Kinetic Studies on the Uptake of VDC and CDDP by TA3Ha Cells

Since nuclear DNA is considered to be the main biological target macromolecule for both CDDP [11, 23, 24] and VDC [1], entry of the drug into the cells constitutes an important step in the drug–cell interaction leading to cell death. Thus a time course drug uptake, using a two compartment pharmacokinetic model [25, 26] was investigated. Two sets of data, (C_e, t) and (C_i, t), where C_e and C_i are the extracellular and intracellular (cell-bound) concentrations of the metal in μg/100 million cells, and t is the duration of drug exposure in minutes

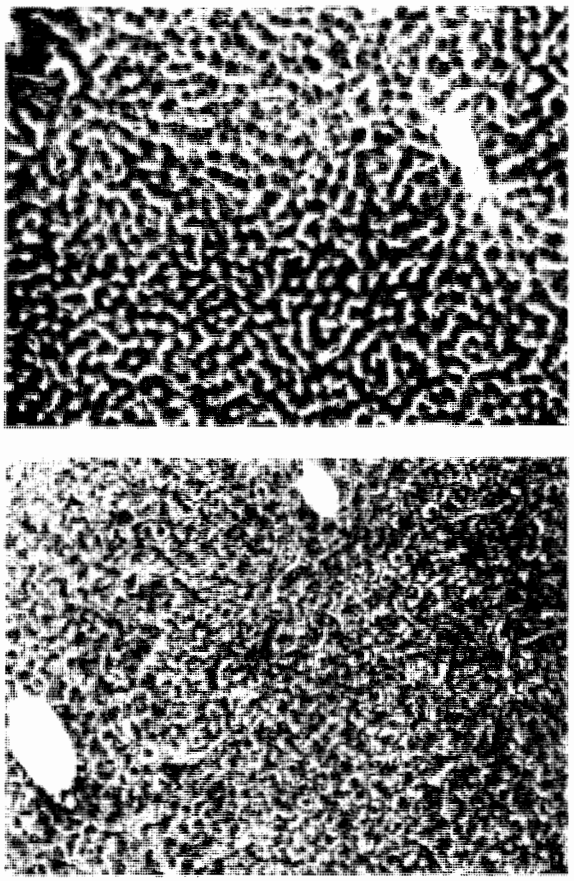

Fig. 5. (a) Histological section of normal mouse liver. ×96. (b) Histological section of mouse liver 5 days after treatment with 60 mg/kg VDC, showing vacuolation of hepatocyte cytoplasm confirmed on fat staining to be fatty degeneration. ×96.

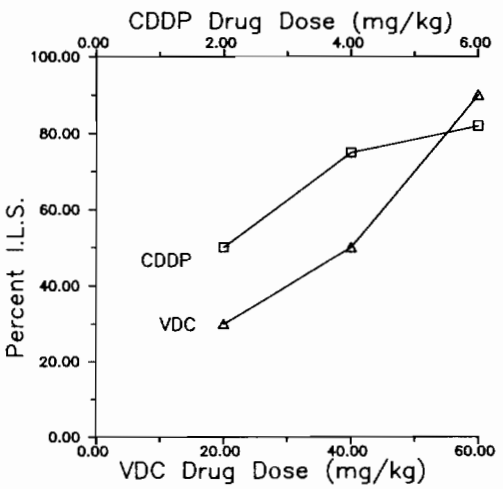

Fig. 6. Comparison of the *in vivo* antitumor effects of vanadocene dichloride (VDC) and *cis*-dichlorodiammineplatinum-(II) (CDDP) against TA3Ha mouse mammary adenocarcinoma in Strain A mice. Results are expressed as increase in life span (%ILS) as a function of drug dosage (mg/kg).

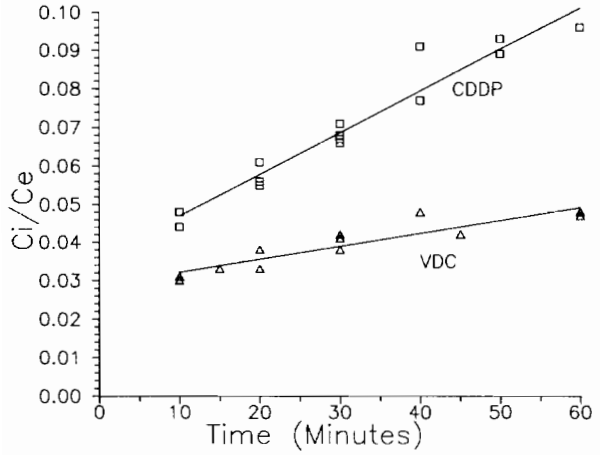

Fig. 7. Rates of uptake of vanadocene dichloride (VDC) and *cis*-dichlorodiammineplatinum(II) (CDDP) by TA3Ha mouse mammary adenocarcinoma cells. Data are expressed as intracellular/extracellular metal concentration ratios as a function of cell exposure time to the drug.

were obtained. The rate law for the drug uptake thus obeys the differential equation:

$$dC_i/dt = k_1C_e - k_2C_i$$

where k_1 and k_2 are transmembrane transfer rate constants for drug entry and efflux, respectively. Since $C_e \gg C_i$ in the present experiments and since plots of drug uptake *versus* C_e are linear in the initial stages (slope = 0.03; $r = 0.999$), it is reasonable to assume that the efflux of the drug is negligible, *i.e.*, $k_2 \approx 0$. Hence, integration yields

$$C_i/C_e = k_1t + \text{constant}$$

and k_1 can be determined by plotting C_i/C_e *versus* t (Fig. 7). A VDC concentration of 118 μg/ml was used in these studies because it approximates the vanadium serum level observed after an i.p. injection of 80 mg/kg VDC into Strain A mice [14]. In the first 60 min of exposure of the TA3Ha cells to VDC, $k_1 = 3.3 \pm 1.1 \times 10^{-4}$ min^{-1} ($r = 0.960$). Under the same conditions with CDDP at 43.2 μg/ml, $k_1 = 12 \pm 2.0 \times 10^{-4}$ min^{-1} ($r = 0.966$). Thus, the rate of TA3Ha cellular uptake (binding) of CDDP is approximately four times that of VDC.

Discussion

This study documents a number of important aspects of vanadocene dichloride antineoplastic activity *vis-à-vis* that of CDDP, a well-established and potent anticancer drug. Our results demonstrate that VDC exerts impressive cytotoxic effects against murine TA3Ha cells as well as against human HEp-2

TABLE III. Comparison of VDC and CDDP Chemical and Biological Properties

Parameter	$(C_5H_5)_2VCl_2$	Reference	*cis*-$Pt(NH_3)_2Cl_2$	Reference
Half-life for first chloride loss (min)	too rapid to measure	12	155	11
Half-life for second chloride loss (min)	24.1	12	890	11
Equilibrium constant for first chloride loss (mM)	too large to measure	12	4.4	11
Equilibrium constant for second chloride loss (mM)	2.7	12	1.9	11
pK_a of first bound H_2O molecule	4.7	12	5.6	11
pK_a of second bound H_2O molecule	5.2	12	7.3	11
Half-life of metal–C_5H_5 or NH_3 bond (h)	>240	12	very large	11
Preferred nucleotide binding sites	H_2O, phosphate	13	N-7 of 5′-dGMP N-1, N-7 of 5′-dAMP N-3 of 5′-dCMP	8–11
Lifetime of metal–nucleotide complex	ms	13	many h	8–11
Cytotoxicity				
HEp-2 *in vitro* (D_0)	0.53 μg/ml		0.46 μg/ml	
TA3Ha *in vivo* (%ILS)	90	[a]	83	[a]
Transmembrane				
transfer constant TA3Ha cells (min^{-1})	$3.3 \pm 1.1 \times 10^{-4}$	[a]	$12 \pm 2.0 \times 10^{-4}$	[a]
Toxicity				
Kidney	none noted	[a]	toxic	8
Small intestine	none noted	[a]	toxic	8
Liver	toxic	[a]	none noted	8

[a]This work.

tumor cells. At non-lethal dose levels, VDC also exhibits antitumor effects against the i.p. implants of TA3Ha tumors. These findings together with those reported by Köpf and Köpf-Maier [1, 4] demonstrate that VDC is active against a wide spectrum of experimental tumors. The present results reveal that the cytotoxicity of VDC against HEp-2 cells ($D_o = 0.530 \pm 0.005$ μg/ml) as well as its effect against the TA3Ha ascites tumors (%ILS, 90) are comparable to those of CDDP ($D_o = 0.46 \pm 0.08$ μg/ml; %ILS, 83). Our drug uptake studies show that VDC enters/binds to the TA3Ha cells approximately four times less rapidly than CDDP. Combined with the above VDC/CDDP cytotoxicity results, the present uptake data suggest that once VDC enters/binds to the TA3Ha cell, it is as much as four times more cytotoxic than CDDP.

The present quantitative histological studies, the blood urea nitrogen level determinations, as well as the serum creatinine levels in mice injected with VDC demonstrate that nephrotoxicity is not a major manifestation of VDC-induced toxicity. Similarly, a lack of alteration in the s/v of jejunal villi in mice treated with VDC demonstrates that gastrointestinal toxicity is also unlikely to be important. The renal and gastrointestinal toxicities following multiple administration of VDC requires further investigation.

The decreased levels of blood urea nitrogen observed for up to three days after VDC administration and the intracytoplasmic lipid droplets in the liver parenchyma indicate that hepatotoxicity may be important in the case of VDC. Recent studies by Köpf-Maier and Funke-Kaiser [27], reported as this work was being completed, show that $(C_5H_5)_2TiCl_2$ administration causes no kidney damage in mice, but does induce symptoms of hepatotoxicity. It will, however, be important to further investigate serum enzyme levels and bilirubin level as well as ultrastructural alterations in the liver tissue.

As already noted, VDC aqueous chemistry and coordination chemistry with DNA constituents is radically different from that of CDDP [11–13]. As summarized in Table III, the former suffers rapid chloride hydrolysis (predominantly to $Cp_2V(OH)_2$ at physiological pH) and exhibits labile, preferential binding to 'hard' oxygen donor ligands. On the other hand, CDDP undergoes slow chloride hydrolysis and preferentially interacts in a nonlabile fashion with 'soft' nitrogen donor ligands (e.g., N-7 of guanine). Given these chemical dissimilarities, it is not surprising that VDC/CDDP biodistribution [14], pharmacokinetic [14], toxicity, and cell uptake patterns are different. That these two complexes exhibit cytotoxicity and antineoplastic behavior which is at all similar is, however, remarkable. Even assuming that DNA is the major biological target for both drugs, it is difficult to rationalize how the mechanisms of action can be similar.

In conclusion, VDC exerts impressive cytotoxic effects and very likely by mechanisms different from those of CDDP. Renal or gastrointestinal toxicity does not appear to be dose limiting for VDC, but hepatotoxicity may be. These findings suggest that VDC is a promising new antitumor agent which may be useful as a single agent or as a component in combination chemotherapy with well-established antitumor agents such as CDDP.

Acknowledgements

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References

1 H. Köpf and P. Köpf-Maier, *Am. Chem. Soc. Symp. Ser., 209*, 315 (1983).
2 P. Köpf-Maier, W. Wagner, B. Hesse and H. Köpf, *Eur. J. Cancer, 17*, 663 (1981).
3 P. Köpf-Maier, B. Hesse, R. Vöightlander and H. Köpf, *Eur. J. Cancer Res. Clin. Oncol., 97*, 31 (1980).
4 (a) P. Köpf-Maier, A. Moormann and H. Köpf, *Eur. J. Cancer Clin. Oncol., 21*, 853 (1985); (b) P. Köpf-Maier and H. Köpf, *Chem. Rev., 87*, 1137 (1987).
5 P. Köpf-Maier and D. Krahl, *Chem.-Biol. Interact., 44*, 317 (1983).
6 P. Köpf-Maier and H. Köpf, *Naturwissenschften, 67*, 415 (1980).
7 P. Köpf-Maier, W. Wagner and H. Köpf, *Naturwissenschaften, 68*, 272 (1981).
8 C. F. J. Barnard, M. J. Cleare and P. C. Hydes, *Chem. Br., 22*, 1001 (1986).
9 B. Rosenberg, *Cancer, 55*, 2303 (1985).
10 M. P. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus Nijhoff, The Hague, Netherlands, 1984.
11 S. J. Lippard, *Science, 218*, 1075 (1982).
12 J. H. Toney and T. J. Marks, *J. Am. Chem. Soc., 107*, 947 (1985).
13 (a) J. H. Toney, C. P. Brock and T. J. Marks, *J. Am. Chem. Soc., 108*, 7263 (1986); (b) J. H. Toney, L. N. Rao, M. S. Murthy and T. J. Marks, *Breast Cancer Res. Treat., 27*, 279 (1985).
14 J. H. Toney, M. S. Murthy and T. J. Marks, *Chem.-Biol. Interact., 56*, 45 (1985).
15 M. S. Murthy, J. H. Toney, L. N. Rao, L. Y. Kuo and T. J. Marks, *77th Annual American Association for Cancer Research Meeting*, Los Angeles, Calif., May 7 - 10, 1986, Abstr. 1107.

16 T. S. Hauschka, L. Weiss, B. A. Holdridge, T. L. Cudney, M. Zumpft and J. A. Planinsek, *J. Nat. Cancer Inst., 47*, 343 (1971).
17 M. S. Murthy, J. D. Travis and E. F. Scanlon, *J. Surg. Oncol., 35*, 44 (1987).
18 M. S. Murthy, H. N. Keer, J. D. Travis, J. D. Cohen, J. A. Mollick, J. D. Khandekar and E. F. Scanlon, *Cancer Res., 44*, 3836 (1984).
19 M. S. Murthy, J. D. Travis, L. C. Erickson, J. D. Khandekar and E. F. Scanlon, *Cancer Res., 45*, 6232 (1985).
20 G. R. Gale, L. M. Atkins and E. M. Walker, Jr., *Am. Clin. Lab. Sci., 12*, 345 (1982).
21 M. S. Murthy, L. N. Rao, J. D. Khandekar and E. F. Scanlon, *Cancer Res., 47*, 774 (1987).
22 M. S. Dunnill and R. Whitehead, *J. Clin. Pathol., 25*, 243 (1972).
23 J. J. Roberts and A. J. Thompson, *Prog. Nucleic Acids Res. Molec. Biol., 22*, 71 (1978).
24 L. A. Zwelling, T. Anderson and K. W. Kohn, *Cancer Res., 39*, 365 (1979).
25 D. I. Goldman in B. Chabner (ed.), 'Pharmacologic Principles of Cancer Treatment', W. B. Saunders, Philadelphia, 1982, pp. 15–44.
26 J. M. Collins and R. L. Dedrick, in B. Chabner (ed.), 'Pharmacologic Principles of Cancer Treatment', W. B. Saunders, Philadelphia, 1982, pp. 77–99.
27 P. Köpf-Maier and P. Funke-Kaiser, *Toxicology, 38*, 81 (1986).