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Antineoplastic Activity and Toxicity of an Organometallic Complex of Ruthenium(II) in Comparison with *cis*-PDD in Mice Bearing Solid Malignant Neoplasms*

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Abstract—*The antineoplastic activity of an organometallic complex of ruthenium(II),* [cis-$RuCl_2(DMSO)_4$]°, *has been examined in comparison with that of* cis *-PDD, using three metastasizing tumors of the mouse: Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma.* [cis-$RuCl_2(DMSO)_4$]° *significantly reduces primary tumor growth in all the tumors tested, and its activity is similarly pronounced at three different dosages in mice bearing Lewis lung carcinoma. On the contrary, the survival time of animals having i.v. or i.m. tumor implants are only moderately increased, and also in the case of combined treatments with surgery. The antineoplastic activity of* cis-*PDD appears to be less pronounced than that of* [cis-$RuCl_2(DMSO)_4$]°, *and is limited to mice bearing B16 melanoma, which, among the three tumors used, appears to be naturally more responsive to* cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]°. *The use of* [cis-$RuCl_2(DMSO)_4$]° *appears advantageous over that of* cis-*PDD since, unlike* cis-*PDD, its antineoplastic effects have been obtained at dosages with reduced host toxicity, indicated by the absence of significant hematological toxicity and toxicity for normal proliferating tissues.*

INTRODUCTION

THE USE of *cis*-dichlorodiammine platinum(II) (*cis*-PDD) as the first organometallic complex useful in the chemotherapeutic treatment of human malignant neoplasms is due to a fortunate serendipitous discovery. Following the observation that the application of an electric current by means of platinum electrodes in a medium containing ammonium chloride was capable of causing inhibition of cell division in bacteria [1], Rosenberg *et al.* showed that *cis*-PDD was produced and was responsible for this finding. *cis*-PDD was later shown to possess marked antitumor activity in numerous experimental tumor systems [2] and to be highly active against selected human neoplasms [3]. Several platinum derivatives have been subsequently synthesized and tested for antitumor activity in an attempt to obtain compounds more active than *cis*-PDD (for examples see references [4–6]); some interesting platinum complexes thus resulted which have been examined in preliminary clinical trials. The therapeutic efficacy of platinum complexes, however, is limited by host toxicity, particularly evident in bone marrow and kidney, as well as in many instances by low water solubility (for a review see ref. [7]).

A reduced number of complexes of other transition metals appears to have been examined for antitumor activity in animal systems [8]. Organometallic complexes of rhodium(I), iridium(I) and ruthenium(II) have been shown to possess, in analogy with *cis*-PDD, antiviral [9], antibacterial [10] and antineoplastic activity [11–14]. The antitumor action of a hydrosoluble organometallic complex of ruthenium(II), *cis*-dichlorotetrakisdimethylsulphoxide ruthenium(II) ([*cis*-$RuCl_2(DMSO)_4$]°), is as pronounced as that of *cis*-PDD on Ehrlich ascites carcinoma and L1210 leukemia [12], and on primary tumor

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growth as well as on the formation of spontaneous and artificial lung metastases in mice bearing Lewis lung carcinoma [14]; at the same time [*cis*-$RuCl_2(DMSO)_4$]° has a reduced toxicity on kidney and intestinal mucosa [12].

The aim of the present investigation is therefore that of examining the dose-dependency of the antitumor activity of [*cis*-$RuCl_2(DMSO)_4$]° and *cis*-PDD in mice bearing Lewis lung carcinoma and the width of the spectrum of responding tumors. The tumor panel used consists of, besides Lewis lung carcinoma, two other murine metastasizing tumors, B16 melanoma and a spontaneous mammary carcinoma of CBA mouse (MCa mammary carcinoma) [15]. The differential effects on primary tumor and on spontaneous and artificial lung metastases have been determined; preoperative chemotherapy was also combined with surgical removal of primary tumor. In addition, the toxicity for normal proliferating tissues was evaluated determining the fractional incorporation of [^{3}H]-dThd into the DNA of the cells, and the hematological toxicity of the tested complexes.

MATERIALS AND METHODS

Drugs and animal treatment

cis-Dichlorotetrakisdimethylsulphoxide ruthenium(II), [*cis*-$RuCl_2(DMSO)_4$]°, was prepared following already reported procedures [16]. *cis*-Dichlorodiammine platinum(II) (*cis*-PDD) was kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, U.S.A.

The animals received i.p. 0.1 ml/10 g body wt of freshly prepared solutions of [*cis*-$RuCl_2(DMSO)_4$]° and *cis*-PDD in isotonic sodium chloride.

Tumor transplantation and evaluation

The Lewis lung carcinoma and B16 melanoma lines used were originally obtained from the National Cancer Institute. These tumors are maintained by implanting 10^6 single viable tumor cells s.c. in the axillary region of C57BL/6 mice purchased from Charles River (Calco, Como, Italy). MCa mammary carcinoma, spontaneously arisen in an old multiparous CBA T6T6 mouse [13], was obtained from the Department of Experimental Biology and Medicine of Rudjer Boskovic Institute, Zagreb, Yugoslavia, and was maintained by transplanting 10^6 single viable tumor cells i.m. into the calf of the left hind leg of CBA mice of our conventional breeding colony. The single-cell suspensions were prepared using tumors obtained from donors similarly inoculated 2 weeks previously. The tumor, freed of capsule and necrotic parts, was minced with scissors, gently forced through a 2.00 × 38-mm disposable needle and suspended in 20 ml of Dulbecco's phosphate-buffered saline. The cell suspension was filtered through a double layer of gauze and washed with buffered saline by centrifugation at 500 *g* for 10 min. Cell viability, as determined by trypan blue exclusion, was at least 45–50%.

When the effects of drug treatment were examined, Lewis lung carcinoma and B16 melanoma were transplanted in BDF1 hybrids; syngeneic female CBA mice were used for transplantation of MCa mammary carcinoma. Primary tumor weight was determined by caliper measurements at the end of treatment, taking the tumor density equal to 1, as the volume of the rotation ellipsoid having short and long axes of A and B respectively:

$$\text{tumor weight} = \pi/6 \times A^2 \times B. \qquad (1)$$

The number of metastases of Lewis lung carcinoma implanted s.c. was determined at termination by examining the surface of the lungs by means of a low-power stereo microscope. The weight of metastases was determined as the sum of their individual weights calculated according to equation (1).

Artificial metastases were obtained by i.v. injection of 0.1 ml per mouse of the tumor cell suspension.

For surgical experiments a radical surgical amputation of the whole tumor-bearing leg was performed in mice bearing i.m. tumors, anesthetized with Ketalar, 125 mg/kg i.p. After a complete incision of the skin all around the upper thigh, the femoral and circumflex arteries were ligated using a synthetic absorbable suture. The femur and muscles were cut with heavy scissors and the wound was closed by pulling the skin onto the stump and clamping it with a silk sterile suture. Contact between blood leaking from the tumor and normal adjacent tissue was avoided during amputation. Tumor weight was determined as the difference between the weight of each tumored leg and the corresponding value obtained with untumored controls having the same body weight.

Hematological toxicity

The number of erythrocytes, leucocytes and platelets was measured in the arterial blood collected from the left ventricle in open-chested mice anesthetized with ethyl urethane (1.5 g/kg), following standard procedures, using a Coulter Counter Mod. ZF.

Measurement of fractional incorporation

The fractional incorporation of [^{3}H]-dThd in cells of bone marrow, spleen and intestinal mucosa (^{3}H in DNA at 1 hr/total ^{3}H in the tissue) was determined by the methods of Houghton and Taylor [17].

RESULTS

The effects of the administration of three different dosages of *cis*-PDD and [*cis*-$RuCl_2(DMSO)_4$]° to mice bearing s.c. Lewis lung carcinoma are reported in Table 1. The higher dosages used for each compound are equitoxic and are equal to the $LD_{0.05}$ determined by the method of Litchfield and Wilcoxon [18] in normal BDF1 mice treated with the same schedule (daily i.p. injections for 14 consecutive days). The effects caused by [*cis*-$RuCl_2(DMSO)_4$]° consist of a marked and similar reduction of primary tumor growth and of spontaneous lung metastasis formation, at all the dosages tested. On the contrary, *cis*-PDD only at its higher dosage causes a significant and similar inhibition of primary tumor growth and of metastasis formation, whereas a marked and significant inhibition limited to spontaneous lung metastases is observed at the next lower dose-level; *cis*-PDD is inactive at the lowest dosage used (Table 1).

The highest dosages used above have been employed also to study the effects of *cis*-PDD and of [*cis*-$RuCl_2(DMSO)_4$]° on primary tumor growth and on the survival time of mice bearing i.m. Lewis lung carcinoma, as well as B16 melanoma and MCa mammary carcinoma. Data reported in Table 2 show that Lewis lung carcinoma grows faster after i.m. implantation and that the effects of the treatment with the tested drugs on primary tumor are less pronounced compared with s.c. tumors. [*cis*-$RuCl_2(DMSO)_4$]° causes statistically significant effects on primary tumor growth in all of the tumor models employed and it significantly increases the survival time of the treated animals with the exception of those bearing B16 melanoma. *cis*-PDD is ineffective on i.m. growth of these tumors and causes a statistically significant prolongation of the survival time only in mice bearing B16 melanoma (Table 2).

Artificial lung metastases obtained by i.v. implantation of tumor cells were less sensitive to drug treatment than spontaneous metastases and primary tumors (Table 3). [*cis*-$RuCl_2(DMSO)_4$]° causes a statistically significant increase of the life-span of mice bearing B16 melanoma and MCa mammary carcinoma and is ineffective in mice bearing Lewis lung carcinoma; *cis*-PDD is inactive in all the three tumors used (Table 3).

The effects of the treatment with *cis*-PDD or [*cis*-$RuCl_2(DMSO)_4$]° combined with surgery on survival time are reported in Table 4. Surgery alone is not curative in mice bearing Lewis lung carcinoma and MCa mammary carcinoma, whereas it cures one out of 13 animals (7.7%) bearing B16 melanoma. The preoperative treatment with [*cis*-$RuCl_2(DMSO)_4$]° significantly increases the survival time of the treated animals and causes limited effects in Lewis lung carcinoma; for B16 melanoma 36% of the treated animals are cured. The treatment with *cis*-PDD causes a statistically significant increase in the lifespan of mice bearing B16 melanoma and 20% of cures and fails to produce significant effects on the two other tumors employed (Table 4).

The hematological toxicity of *cis*-PDD in

Table 1. Effects of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on primary tumor growth and on the formation of spontaneous pulmonary metastases in mice bearing s.c. Lewis lung carcinoma*

Compound	Daily dose (mg/kg)	Primary tumor weight* (mg)	Lung metastases† Total No.	Lung metastases† Weight (mg)
Controls		100 ± 10 (773.3 ± 74.4)‡	100 ± 12 (21.1 ± 2.5)‡	100 ± 16 (77.7 ± 12.2)‡
cis-PDD	0.13	91 ± 9	117 ± 24	84 ± 16
	0.26	111 ± 17	54 ± 11§	41 ± 12§
	0.52	43 ± 6§	30 ± 8§	25 ± 10§
[*cis*-$RuCl_2(DMSO)_4$]°	152	47 ± 9§	55 ± 8§	39 ± 9§
	305	54 ± 8§	73 ± 21	53 ± 15§
	610	32 ± 5§	36 ± 11§	21 ± 8§

*Determined at the end of treatment on day 15.

†Determined at termination on day 21 from tumor implantation.

‡Actual finding in the control group.

§Mean significantly different from that of controls; Student-Newmann-Keuls test [19], $P = 0.05$.

Each value is the mean percentage ratio (treated over controls) ± S.E. obtained in groups of 8–10 animals (16 controls) implanted s.c. with Lewis lung carcinoma on day 0 (10^6 tumor cells/mouse) and treated i.p. daily on days 1–14 after tumor implantation.

Table 2. Effects of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on primary tumor growth and on the survival time of mice bearing i.m. Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma*

Tumor line	Treatment Compound	Daily dose (mg/kg)	Primary tumor weight* (mg)	Survival time (days)
Lewis lung carcinoma	Controls		100 ± 15	100 ± 8
			(2464 ± 378)†	(23.6 ± 1.9)†
	cis-PDD	0.52	69 ± 27	98 ± 8
	[*cis*-$RuCl_2(DMSO)_4$]°	610	60 ± 6‡	125 ± 7‡
B16 melanoma	Controls		100 ± 13	100 ± 7
			(1216 ± 162)†	(23.1 ± 1.6)†
	cis-PDD	0.52	76 ± 14	124 ± 10‡
	[*cis*-$RuCl_2(DMSO)_4$]°	610	37 ± 9‡	109 ± 4
MCa mammary carcinoma	Controls		100 ± 5	100 ± 11
			(1525 ± 76)†	(29.1 ± 3.1)†
	cis-PDD	0.52	104 ± 10	94 ± 7
	[*cis*-$RuCl_2(DMSO)_4$]°	610	55 ± 13‡	125 ± 9‡

*Determined at the end of treatment on day 14 from tumor implantation.
†Actual findings in control groups.
‡Mean significantly different from that of the relevant controls; Student–Newmann–Keuls test [19], $P = 0.05$.
Each value is the mean percentage ratio (treated over controls) ± S.E. obtained in groups of at least 8 animals implanted i.m. with the tumors on day 0 (10^6 tumor cells/mouse) and treated i.p. daily on days 1–14 after tumor implantation.

Table 3. Effects of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on the survival time of mice with artificial metastases obtained from Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma*

Tumor line	Treatment Compound	Daily dose (mg/kg)	Survival time (days)
Lewis lung carcinoma	Controls		100 ± 3
			(18.0 ± 0.5)*
	cis-PDD	0.52	106 ± 4
	[*cis*-$DuCl_2(DMSO)_4$]°	610	109 ± 4
B16 melanoma	Controls		100 ± 2
			(29.8 ± 0.5)*
	cis-PDD	0.52	102 ± 2
	[*cis*-$RuCl_2(DMSO)_4$]°	610	116 ± 2†
MCa mammary carcinoma	Controls		100 ± 4
			(21.5 ± 0.9)*
	cis-PDD	0.52	111 ± 5
	[*cis*-$RuCl_2(DMSO)_4$]°	610	132 ± 10†

*Actual finding in the control groups.
†Mean significantly different from that of the relevant controls; Student–Newmann–Keuls test [19], $P = 0.05$.
Each value is the mean percentage ratio (treated over controls) ± S.E. obtained in groups of at least 8 animals implanted i.v. with the tumors on day 0 (2.5×10^5 tumor cells/mouse; 2.0×10^5 for B16 melanoma) and treated i.p. daily on days 1–8 after tumor transplantation.

normal mice repeatedly treated for 11 consecutive days consists of a statistically significant reduction in the number of leucocytes and thrombocytes, evident at the end of treatment and persisting, in the case of platelets, for 1 week. [*cis*-$RuCl_2(DMSO)_4$]° is completely devoid of such effects but causes a slight increase of the leucocyte number measured 1 week after the end of treatment (Table 5); no alteration in the leucocyte differential counts is correspondingly observed (unreported results).

The toxicity of *cis*-PDD for normal tissues such as bone marrow, spleen and intestinal mucosa is also evident. The administration of a single therapeutically active dosage of *cis*-PDD to normal BDF1 mice significantly reduces the fractional incorporation of [^{3}H]-dThd into the DNA of the tissues examined 24 hr after drug treatment. The effects on fractional incorporation of *cis*-PDD at 48 hr and those of [$RuCl_2(DMSO)_4$]° at 24 and 48 hr after treatment are not statistically significant (Fig. 1).

Table 4. Effects of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on primary tumor growth and on the survival time of mice bearing i.m. Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma, undergoing surgical amputation of primary tumor at the end of treatment*

Tumor line	Treatment: Compound	Daily dose (mg/kg)	Primary tumor weight* (g)	Survival time (days)	Cures†
Lewis lung carcinoma	Controls		100 ± 5	100 ± 5	0/21
			(2.06 ± 0.1)‡	(27.5 ± 1.3)‡	
	cis-PDD	0.52	90 ± 5	103 ± 4	0/23
	[*cis*-$RuCl_2(DMSO)_4$]°	610	71 ± 5§	116 ± 9§	0/14
B16 melanoma	Controls		100 ± 6	100 ± 8	1/13
			(1.79 ± 0.1)‡	(40.3 ± 3.1)‡	
	cis-PDD	0.52	34 ± 11§	134 ± 8§	2/10
	[*cis*-$RuCl_2(DMSO)_4$]°	610	31 ± 6§	126 ± 8§	4/11
MCa mammary carcinoma	Controls		100 ± 16	100 ± 4	0/19
			(2.53 ± 0.4)‡	(33.6 ± 1.5)‡	
	cis-PDD	0.52	86 ± 8	115 ± 7	0/10
	[*cis*-$RuCl_2(DMSO)_4$]°	610	57 ± 8§	130 ± 5§	0/16

*Determined at amputation on day 12 after i.m. tumor implantation.
†Animals surviving at 4 months over the number of surgically treated animals without tumor site regrowth.
‡Actual finding in control groups.
§Mean significantly different from that of the relevant controls; Student–Newmann–Keuls test [19], $P = 0.05$.
Each value is the mean percentage ratio (treated over controls) ± S.E. obtained in groups of at least 10 animals implanted i.m. with the tumors on day 0 (10^6 tumor cells/mouse). The treatment was performed i.p. daily on days 1–11 and surgical amputation of the primary tumor was performed on day 12 after tumor implantation. Animals with tumor site regrowth after surgery did not exceed 12.5% and were excluded from the presented data.

Table 5. Effects of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on the number of blood cells in normal BDF1 mice*

Days from the end of treatment	Compound	Daily dose (mg/kg)	Erythrocytes ($\times 10^9$/ml)	Leucocytes ($\times 10^6$/ml)	Platelets ($\times 10^6$/ml)
	Controls		8.24 ± 0.48	4.02 ± 0.43	0.96 ± 0.08
1	*cis*-PDD	0.52	7.64 ± 0.22	2.96 ± 0.57*	0.68 ± 0.11*
	[*cis*-$RuCl_2(DMSO)_4$]°	610	7.21 ± 0.47	5.41 ± 0.93	0.74 ± 0.09
	Controls		8.19 ± 0.35	4.56 ± 0.85	1.00 ± 0.04
8	*cis*-PDD	0.52	7.79 ± 0.20	4.10 ± 0.77	0.62 ± 0.02*
	[*cis*-$RuCl_2(DMSO)_4$]°	610	7.92 ± 0.25	7.92 ± 0.25*	0.91 ± 0.02

*Mean significantly different from that of the relevant controls; Student–Newmann–Keuls test [19], $P = 0.05$.
Each value is the mean ± S.E. obtained in groups of 6–9 BDF1 mice treated i.p. for 11 consecutive days.

DISCUSSION

The antitumor activity of [*cis*-$RuCl_2(DMSO)_4$]° is evident against the i.m. growth of the three tumors presently examined and also in s.c. Lewis lung carcinoma at all the three dose-levels tested; in animals with s.c. Lewis lung carcinoma [*cis*-$RuCl_2(DMSO)_4$]° is also effective in significantly reducing the development of spontaneous pulmonary metastases. In spite of these effects, the survival time of mice bearing i.m. tumor implants is marginally increased in the case of Lewis lung carcinoma and MCa mammary carcinoma, whereas it is unaffected in the case of B16 melanoma. Similarly, a marginal increase of survival time is observed in mice with i.v. tumor implants with the exception of Lewis lung carcinoma, where no life-span prolongation is noted. When the treatment of mice bearing i.m. tumor implants precedes surgical removal of primary tumors, a significant increase of survival time is observed. Noteworthy is the fact that, although surgery alone cured one out of 13 animals (7.7%), four out of 11 (36%) mice bearing B16 melanoma were cured by preoperative treatment with [*cis*-$RuCl_2(DMSO)_4$]°. The antitumor effects of *cis*-PDD appear less pronounced than those of [*cis*-$RuCl_2(DMSO)_4$]°. Indeed, in s.c. Lewis lung carcinoma they are evident in a narrower dose-range, and as far as i.m. implants of the three tumors tested are concerned, they are significant only for B16 melanoma. When *cis*-PDD is administered prior to surgery a number of cures (20%) intermediate between that of controls and that of [*cis*-$RuCl_2(DMSO)_4$]°-treated animals is observed.

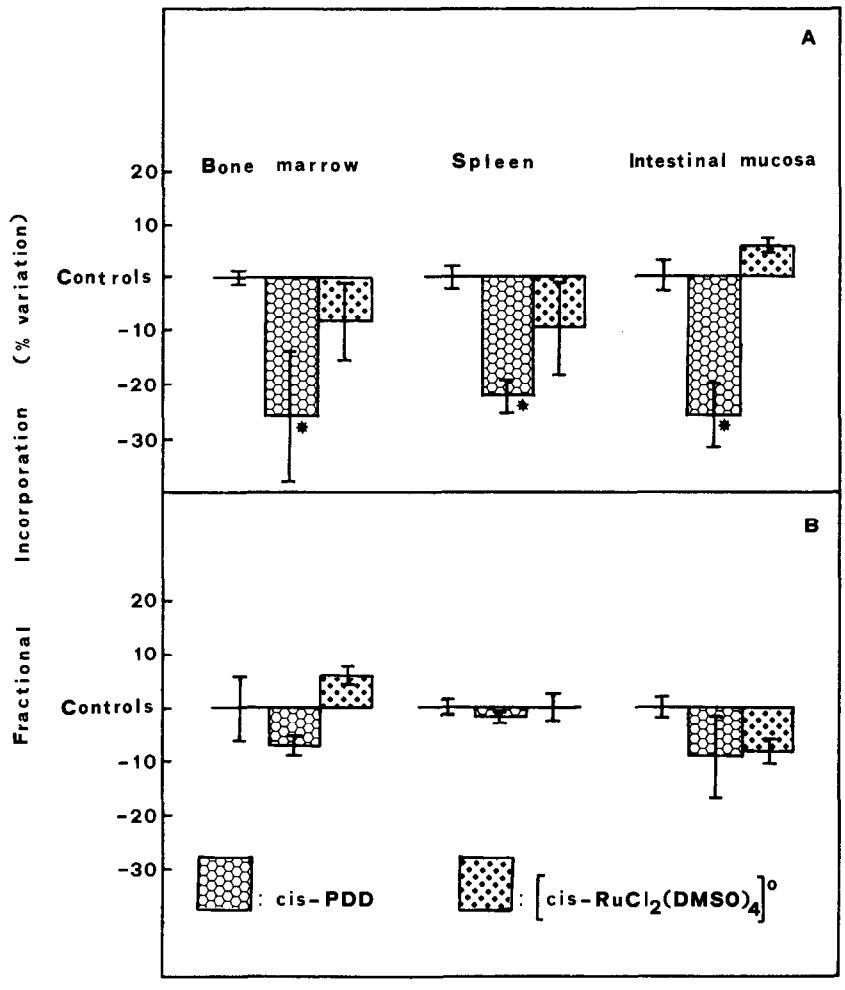

Fig. 1. Effects of a single therapeutically active dose of cis-*PDD and* [cis-$RuCl_2(DMSO)_4$]° *on the fractional incorporation of* [^{3}H]-*dThd in normal tissues. *Mean significantly different from that of the relevant controls; Student–Newmann–Keuls test* [19], P = 0.05. *Each value is the percentage variation* (±*S.E.*) *of the mean of each treated group to that of the relevant controls obtained using* 5 *BDF*1 *mice treated i.p.* 24 (*panel A*) *or* 48 hr (*panel B*) *before the evaluation of the fractional incorporation of* [^{3}H]-*dThd. The dosages used were* 0.52 mg/kg *for* cis-*PDD and* 610 mg/kg *for* [cis-$RuCl_2(DMSO)_4$]°.

Thus the responsiveness of the tumors presently used to the organometallic complexes tested in the different experimental conditions employed is limited; B16 melanoma appears to be naturally more responsive to the effects of both complexes, at least in the most therapeutically meaningful experiments involving surgery. At the same time it appears that the antitumor activity of [*cis*-$RuCl_2(DMSO)_4$]° is at least as pronounced as that of *cis*-PDD. Moreover, the use of [*cis*-$RuCl_2(DMSO)_4$]° also appears to be advantageous over that of *cis*-PDD in terms of reduced toxicity, indicated by the lack of alteration of the parameters presently investigated together with previously published histological data [12].

No evident correlation between antitumor activity and chemical structure or reactivity can be presently found for the rhodium(I), iridium(I) and ruthenium(II) complexes so far examined in the laboratory of the authors [12–14]. On the other hand, the data presented above encourage the synthesis and characterization of the chemical and antitumor properties of organometallic complexes of other transition metals that have various configurations which is presently underway, aiming to find still more advantageous analogs of *cis*-PDD.

REFERENCES

1. Rosenberg B, Van Camp L, Krigas T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature* 1965, **205**, 698–699.

2. Rosenberg B, Van Camp L, Trosko JE, Mansour VH. Platinum compounds: a new class of potent antitumor agents *Nature* 1969, **222,** 385–386.
3. Prestayko AW, D'Aoust JC, Issell BF, Crooke ST. Cis-platin (*cis*-dichlorodiammino-platinum(II)). *Cancer Treat Rev* 1979, **6,** 17–39.
4. Braddock PD, Connors TA, Jones M, Khokhar AR, Melzack DH, Tobe ML. Structure activity relationships of platinum complexes with antitumor activity. *Chem Biol Interact* 1975, **11,** 145–161.
5. Speer RJ, Storey CJ, Hall LM, Ridgway HJ. Relative therapeutic efficacy of platinum complexes against advanced B16 melanoma in mice. *J Clin Hematol Oncol* 1981, **11,** 47–53.
6. Rose WC, Schuring JE, Huftalen JB, Bradned WT. Antitumor activity and toxicity of cisplatin analogs. *Cancer Treat Rep* 1982, **66,** 135–146.
7. Hill JM, Speer RJ. Organo-platinum complexes as antitumor agents. *Anticancer Res* 1983, **2,** 173–186.
8. Cleare MJ. Transition metal complexes in cancer chemotherapy. *Coord Chem Rev* 1974, **12,** 349–405.
9. Monti-Bragadin C, Pani B, Cantini M, Giraldi T, Mestroni G, Zassinovich G. Effetti antivirali di un complesso metallo-organico di Rh(I). *G Ital Chemioter* 1974, **21,** 109–112.
10. Monti-Bragadin C, Giraldi T, Cantini M, Zassinovich G, Mestroni G. Inhibition of bacterial growth and nucleic acid synthesis by planar complexes of rhodium(I). *FEBS Lett* 1974, **43,** 13–16.
11. Giraldi T, Zassinovich G, Mestroni G. Antitumor action of planar organometallic rhodium(I) complexes. *Chem Biol Interact* 1974, **9,** 389–394.
12. Giraldi T, Sava G, Bertoli G, Mestroni G, Zassinovich G. Antitumor action of two rhodium and ruthenium complexes in comparison with *cis*-dichlorodiammine platinum(II). *Cancer Res* 1977, **37,** 2662–2666.
13. Giraldi T, Sava G, Mestroni G, Zassinovich G, Stolfa D. Antitumor action of rhodium(I) and iridium(I) complexes. *Chem Biol Interact* 1978, **22,** 231–238.
14. Sava G, Giraldi T, Mestroni G, Zassinovich G. Antitumor effects of rhodium(I), iridium(I) and ruthenium(II) complexes in comparison with *cis*-dichlorodiammine platinum(II) in mice bearing Lewis lung carcinoma. *Chem Biol Interact* 1983, **45,** 1–6.
15. Poljak-Blazi M, Boranić M, Marzan B, Radačić M. A transplantable aplastic mammary carcinoma of CBA mice. *Vet Arhiv* 1981, **51,** 99–107.
16. Evans IP, Spencer A, Wilkinson G. Dichlorotetrakis (dimethylsulphoxide) ruthenium(II) and its use a source material for new ruthenium(II) complexes. *J Chem Soc* 1973, Dalton, 204–209.
17. Houghton PJ, Taylor DM. Fractional Incorporation of [^{3}H]thymidine and DNA specific activity as assays of inhibition of tumor growth. *Br J Cancer* 1977, **35,** 68–77.
18. Litchfield J, Wilcoxon F. A simplified method of evaluating dose–effect experiments. *J Pharmacol Exp Ther* 1949, **96,** 99–113.
19. Sokal RR, Rohlf FJ. Single classification analysis of variance. In: *Biometry*. San Francisco, CA, W. H. Freeman and Co., 1969, 204–252.