Cancer Chemother Pharmacol (1991) 27: 301–307

034457049100009V

Cancer Chemotherapy and Pharmacology

In vitro evaluation of platinum, titanium and ruthenium metal complexes in cisplatin-sensitive and -resistant rat ovarian tumors

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Received 20 April 1990/Accepted 10 September 1990

Summary. The antitumor activity of eight new metal complexes (three platinum, one titanium, four ruthenium derivatives) was investigated in a cisplatin (DDP) – sensitive (O-342) and a DDP-resistant (O-342/DDP) ovarian tumor line using the bilayer soft-agar assay. A continuous exposure set up at logarithmically spaced concentrations was used to test the drugs; to uncover possible pharmacokinetic features, a short-term exposure was additionally included for selected compounds. DDP served as the reference drug. The following compounds were investigated: 18-crown-6-tetracarboxybis-diammineplatinum(II) (CTDP), *cis*-aminotrismethylenephosphonato-diammineplatinum(II) (ADP), *cis*-diamminecyclohexano-aminotrismethylenephosphonato-platinum(II) (DAP), diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV) (DBT, budotitane), *trans*-imidazolium-bisimidazoletetrachlororuthenate(III) (ICR), *trans*-indazolium-tetrachlorobisindazoleruthenate(III) (IndCR), *cis*-triazolium-tetrachlorobistriazoleruthenate(III) (TCR) and *trans*-pyrazolium-tetrachlorobispyrazoleruthenate(III) (PCR). Of the new metal complexes, CTDP was the most active compound in O-342, resulting in a percentage of control plating efficiency ($\pm$SE) of 1 ± 1, 12 ± 8 and 40 ± 21 following continuous exposure to 10, 1 and 0.1 μM, respectively, and was thus comparable to DDP at equimolar concentrations. In the resistant line, 10 μM CTDP reduced colony growth to $18\% \pm 8\%$, whereas an equimolar concentration of DDP effected a reduction to $26\% \pm 9\%$. During short-term exposure, CTDP was inferior to DDP, which may be ascribed to the stability of the bis-dicarboxylate platinum ring system. The titanium compound DBT, in contrast, showed promising effects at its highest concentration (100 μM) during short-term exposure in both lines; at this concentration the activity in O-342/DDP was higher than that in O-342 ($7\% \pm 7\%$ vs $34\% \pm 17\%$ of control plating efficiency at 100 μM). All ruthenium complexes showed higher activity in the resistant line O-342/DDP than in the sensitive counterpart. ICR was the most active compound. Following continuous exposure of O-342/DDP cells to 10 μM ICR, colony growth was reduced to $18\% \pm 4\%$ that of controls. Further studies should concentrate on CTDP and ICR for the following reasons: the activity of CTDP was equal to that of DDP at equimolar concentrations during continuous exposure; considering that the in vivo toxicity of DDP was 3-fold that of CTDP, an increase in the therapeutic index of CTDP would be expected. ICR showed the best effect of all ruthenium complexes; it was superior to DDP in the resistant line.

Introduction

Cisplatin (*cis*-diamminedichloroplatinum, DDP) is one of the first-line chemotherapeutic agents for the treatment of ovarian carcinoma, testicular carcinoma and head and neck cancer [16]. However, the factors limiting the use of this compound are its strong side effects [15]; in addition,

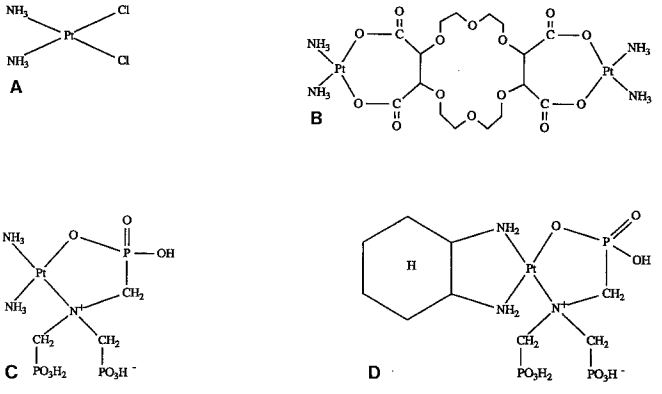

Fig. 1 A–D. Structures of platinum complexes. **A** DDP, *cis*-diamminedichloroplatinum(II); **B** CTDP, 18-crown-6-tetracarboxybis-diammineplatinum(II); **C** ADP, *cis*-aminotrismethylenephosphonato-diammineplatinum(II); **D** DAP, *cis*-diamminecyclohexano-aminotrismethylenephosphonato-platinum(II)

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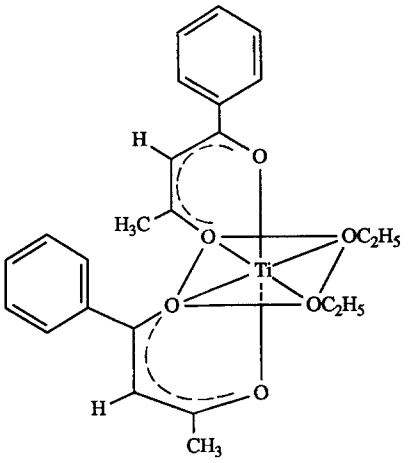

Fig. 2. Structure of the titanium complex DBT, diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV) (budotitane)

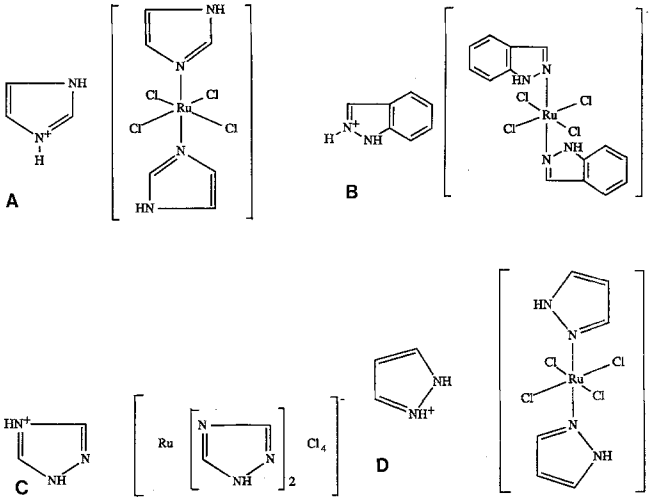

Fig. 3 A–D. Structures of ruthenium complexes. **A** ICR, *trans*-imidazolium-bisimidazoletetrachlororuthenate(III); **B** IndCR, *trans*-indazolium-tetrachlorobisindazoleruthenate(III); **C** TCR, *cis*-triazolium-tetrachlorobistriazoleruthenate(III); **D** PCR, *trans*-pyrazolium-tetrachlorobispyrazoleruthenate(III)

primary and secondary (acquired) resistance are observed [13]. Consequently, there is great interest in obtaining agents that have more favourable therapeutic indices and do not show cross-resistance to DDP. To accomplish this task, several new platinum analogues have been developed in recent years. In addition, current research has been aimed at the development of new metal complexes such as titanium [20] and ruthenium compounds [23].

The present report describes investigations on the in vitro activity of eight new metal complexes as compared with that of DDP. The following platinum analogues (Fig. 1) were investigated: CTDP (18-crown-6-tetracarboxybis-diammineplatinum(II), $[18C6(COO)_4][Pt(NH_3)_2]_2$), a molecule that is similar to carboplatin but contains two platinum atoms; ADP (*cis*-aminotrismethylenephosphonato-diammineplatinum(II), $cis[Pt(ATMP)(NH_3)_2]$), which shows the same activity in rat osteosarcoma as DDP, although its nephrotoxicity is considerably lower [24]; and DAP (*cis*-diamminecyclohexano-aminotrismethylenephosphonato-platinum(II),[cis-[Pt(DACH)(ATMP)]), a 1,2-diammine-cyclohexaneplatinum(II) (DACH) derivative. The latter group has been found to lack cross-resistance to DDP [6, 17]. One titanium complex, DBT (budotitane, diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV), $Ti(bzac)_2(OEt)_2$) (Fig. 2), was also investigated; it is presently undergoing clinical trials. Compounds of this group have been shown to be more active than DDP in experimental autochthonous colorectal carcinomas in rats [5]. The third group investigated were ruthenium complexes (Fig. 3): ICR [*trans*-imidazolium-bisimidazoletetrachlororuthenate(III), $ImH(RuIm_2Cl_4)$], IndCR [*trans*-indazolium-tetrachlorobisindazoleruthenate(III), $IndH(RuInd_2Cl_4)$], TCR [*cis*-triazolium-tetrachlorobistriazoleruthenate(III), *cis*-$TrH(RuTr_2Cl_4)$] and PCR [*trans*-pyrazolium-tetrachlorobispyrazoleruthenate(III), $PzH(RuPz_2Cl_4)$]. Compounds of this group have shown better activity than DDP in various rodent tumors, e.g. leukemia, sarcoma [22] and melanoma [21].

The bilayer soft-agar assay using continuous exposure was chosen for comparison of the activity of the new metal complexes in a DDP-sensitive and a DDP-resistant ovarian tumor line, which are used in our laboratory for the investigation of resistance mechanisms in ovarian tumors [8, 28, 29]. With regard to possible pharmacokinetic differences in the decomposition rate of platinum complexes to their active forms, as well as the susceptibility of DBT to hydrolysis [20], and to study the possible role of activation of Ru(III) ions to the more active Ru(II) ion [9], we additionally employed a short-term incubation (1 h) [18] for the most promising compounds of each group, using the same assay.

Materials and methods

Tumor model

Ovarian tumor 342 (O-342) was induced in a female BDIX rat by a single i.p. injection of 100 mg/kg ethylnitrosourea with a manifestation time of 422 days. Histologically, the primary tumor corresponded to a partly cystic, partly papillary growing granulosa-cell tumor. The tumor was maintained by i.p. inoculation in BDIX rats at an interval of about 10 days. Transplantation of 5 mg tumor tissue per rat resulted in a median survival of 15 days (range, 14–18 days). Resistance of O-342 to DDP was developed in BDIX rats by repeated i.p. injections of this drug (for details see [29]). This tumor was designated O-342/DDP. Following DDP treatment (dose, 1.2 mg/kg ×5), the median survival of rats bearing O-342 and O-342/DDP was 61 and 15 days, respectively (untreated control groups: 17.5 and 15 days, respectively) [29].

Drugs

DDP was purchased from Behringwerke (Marburg, FRG). All new compounds were synthesized and kindly supplied by Dr. Dr. B. K. Keppler, Institute of Inorganic Chemistry, University of Heidelberg (FRG). Purity was determined by elemental analysis, infrared and [^{1}H]-nuclear magnetic resonance spectroscopy. Compounds were dissolved in physiological saline; DBT required the addition of polyvinylpyrrolidone (PVP; 1 : 11, v/v) prior to solution (personal communication, B. K. Keppler). For

Table 1. Number of colonies and plating efficiency in control dishes

Incubation method	Tumor	Mean number of colonies ±SE	Plating efficiency (%) ±SE
Continuous	O-342	611±153	0.12±0.03
	O-342/DDP	2,533±635	0.51±0.13
Short-term	O-342	1,293±694	0.26±0.13
	O-342/DDP	1,523±324	0.3 ±0.06

continuous exposure (CE), final concentrations of 10, 1 and 0.1 μM were prepared. The compounds were incorporated into the top (cellular) layer at the time of plating according to Shoemaker et al. [26]. For short-term exposure (STE), final concentrations of 100, 10 and 1 μM were used in the incubation tubes. Dose levels were chosen on the basis of optimal in vivo doses.

Clonogenic assay

All tumor material was freshly excised prior to plating. A single-cell suspension was obtained by mechanical disaggregation with scissors and subsequent incubation with enzymes (collagenase/dispase 0.1%; Boehringer, Mannheim, FRG) at 37° C for 30 min. The process was stopped with a 0.1% solution of ethylenediamminetetraacetic acid (EDTA). Subsequently, cells were washed and passed through nylon sieves with 200- and 50 μm mesh size to remove any remaining clumps. Cell counts were performed using the Neubauer method and the percentage of viable cells was determined by trypan blue exclusion. A modification of the two-layer soft-agar culture system introduced by Hamburger and Salmon [14] was used. The base layer was prepared 1 day prior to the experiment and consisted of 1 ml McCoy's 5A medium supplemented with 10% fetal calf serum (FCS), 5% horse serum (Gibco, Karlsruhe), sodium pyruvate (0.22 mg/ml), L-serine (42 μg/ml), L-glutamine (2.9 μg/ml), sodium bicarbonate (4.5 mg/ml), penicillin (100 IU/ml), streptomycin (100 μg/ml), asparagine (100 μg/ml) (all from Serva, Heidelberg), DEAE-dextran (375 μg/ml); Pharmacia, Freiburg), tryptic soy broth (7.5 mg/ml; Difco, Detroit) and agar at a final concentration of 0.5% (Bacto agar, Difco). In all, 1 ml suspension was immediately dispensed into a 35-mm petri dish.

Continuous exposure (CE)

The upper layer was prepared for CE culture (>7 days) as follows. The final cell concentration was adjusted to 5.5×10^5 viable cells/ml enriched Basal Medium Eagle (BME). The supplements were 15% horse serum, porcine insulin (2 IU/ml; Hoechst, Frankfurt), ascorbic acid (53 μg/ml; Serva, Heidelberg), penicillin (100 IU/ml), streptomycin (100 μg/ml), DEAE-dextran (250 μg/ml) and asparagine (66 μg/ml). Then, this suspension was divided into aliquots of 2.7 ml, to which 0.3 ml agar (final concentration, 0.3%) and 30 μl drugs were added. Plating was performed by aspirating 1 ml of the resultant upper-layer mixture and dispensing this volume on top of the lower layer. The DDP-resistant ovarian tumor required a concentration of 5×10^{-5} M 2-mercaptoethanol in the upper layer for growth; O-342 did not require this growth factor. Initial experiments showed that a concentration of 5×10^{-5} M 2-mercaptoethanol for O-342 did not alter the test results, thus rendering the drug effects in both tumors comparable.

Short-term exposure (STE)

For short-term exposure (STE, 1 h) 0.5 ml cell suspension (3×10^6 cells/ml) were transferred to tubes containing 0.85 ml McCoy's 5A medium with 10% FCS; drugs were added at a constant volume of 0.15 ml. Control tubes contained the respective solvent concentrations. Cells were incubated for 1 h at 37° C, then centrifuged at 150 *g* for 10 min, washed twice with McCoy's 5A medium containing 10% FCS and prepared for culture as follows. The upper layer was prepared by resuspension of the cell pellets in 2.7 ml enriched BME plus 0.3 ml agar (final concentration, 0.3%). Plating was performed as described above. Since the colony count in control plates of the sensitive tumor dropped below the quality level of 100 due to the more intensive preplating procedures, both tumors additionally received 2-mercaptoethanol (5×10^{-5} M) in the upper layer.

For each experiment, three drug concentrations and a positive reference (mercuric chloride, 100 μM in the upper layer for CE or in the incubation tube for STE) were tested in triplicate (5×10^5 cells/plate). Six vehicle-treated cultures were plated for determination of control growth. Cultures were incubated at 37° C in a humidified atmosphere containing 7.5% CO_2. Control plates were monitored for growth every other day using a stereomicroscope (WILD M8; Leitz, Wetzlar). At the time of maximal colony formation (7–14 days in culture), final colony counts were performed with an automatic image analysis system (40-10 image analysis system, AI-Tektron-Meßsysteme, Meerbusch, FRG; ITC-510 video-camera, Ikegami Electronics Europe, Neuss, FRG). At 1 day prior to final evaluation, all dishes were incubated with 1 ml tetrazolium chloride (1 mg/ml), which stained vital cells only [3]. Objects presenting a circular profile with a minimal diameter of 60 μm were scored as colonies. For quality control purposes, plates were stained with tetrazolium chloride on days 0 and 2 and then frozen at –20° C 1 day later, after the addition of 1 ml glycerin. These plates served as negative controls. Further quality-control parameters were a minimal colony number of 100/plate in the control group, initial colony counts on days 0 or 2 of <20% of the final colony count in the control group and a coefficient of variation of <40% in each group [11]. The positive reference compound mercuric chloride (100 μM in the upper layer) had to effect a colony survival of <20% of control values. Only experiments fulfilling these criteria were included in the evaluation. A compound was considered to be active if colony formation was reduced to ⩽30% of the respective control value.

Results

Table 1 shows the plating efficiencies for the DDP-sensitive (O-342) and -resistant (O-342/DDP) tumors: following CE, plating efficiencies were 0.12%±0.03% and 0.51%±0.13%, respectively and after STE the respective values were 0.26%±0.13% and 0.30%±0.06%. The degree of resistance to DDP was determined using ID_{70} values for DDP in both lines, which were extrapolated from plots using the data from Tables 2 and 3 (plots not given). We found a 28-fold difference between the ID_{70} values for O-342/DDP (8.7 μM) and O-342 (0.31 μM) (Fig. 4) after CE., whereas the ratio of ID_{70} values after STE between O-342/DDP (33.5 μM) and O-342 (11.8 μM) was only 2.8 (Fig. 4).

At the ID_{50} level, a 44-fold difference between O-342 (0.09 μM) and O-342/DDP (4 μM) after CE. and a 4.7-fold difference between O-342 (3.8 μM) and O-342/DDP (18 μM) after STE were observed. Thus, resistance factors in the two incubation models varied by a factor of approximately 10, which could be demonstrated at both the widely used ID_{50} cutoff point and at ID_{70}, the minimal growth-inhibition value required in this assay for a drug to be considered active. Treatment with all platinum compounds caused a dose-dependent decrease in colony formation (Table 2) in both tumor lines. In O-342, their activity was superior to all other non-platinum metal complexes after both CE and STE. DDP and CTDP were the most effective agents among this group. Following CE of O-342, they

Table 2. Continuous exposure: effect of 10, 1 and 0.1 μM treatment on the number of colonies of O-342 and O-342/DDP (percentage of control plating efficiency ± SE)

Compounds	O-342				O-342/DDP			
	10 μM	1 μM	0.1 μM	ID_{70}[a]	10 μM	1 μM	0.1 μM	ID_{70}[a]
Platinum:								
DDP	1± 1	16± 9	43± 7	0.31	26± 9	86±12	106±13	8.7
CTDP	1± 1	12± 8	40±21	0.22	18± 8	92±12	106±10	5.6
ADP	8± 4	23±12	56±11	0.61	67±10	94±15	111± 8	>10
DAP	16± 9	52±10	75±10	4.1	84±17	101± 7	104±30	>10
Titanium:								
DBT	58±10	122±23	79±12	>10	95±21	100±15	91±14	>10
Ruthenium:								
ICR	48± 9	60± 5	91± 5	>10	18± 4	65± 9	101±16	7
IndCR	74± 3	96±10	97± 9	>10	58± 3	96±18	107± 6	>10
TCR	62± 4	76±13	107± 8	>10	59±11	106±13	122±17	>10
PCR	69± 4	88±13	73±30	>10	37± 6	80±11	91±19	>10

[a] Inhibitory dose – 70% values, expressed in μM

Table 3. Short-term exposure: effect of 100, 10 and 1 μM treatment on the number of colonies of O-342 and O-342/DDP (percentage of control plating efficiency ± SE)

Compounds	O-342				O-342/DDP			
	100 μM	10 μM	1 μM	ID_{70}[a]	100 μM	10 μM	1 μM	ID_{70}[a]
Platinum:								
DDP	2± 1	32±14	72±37	11.8	0± 0	63±17	73±13	33.5
CTDP	16± 6	60±21	67±17	49	43±35	83± 7	94± 4	>100
Titanium:								
DBT	34±17	110±42	127±46	>100	7± 7	81±12	73± 9	51
Ruthenium:								
ICR	56±17	134±56	111±26	>100	81±12	84±14	80±16	>100

[a] Inhibitory dose – 70% values, expressed in μM

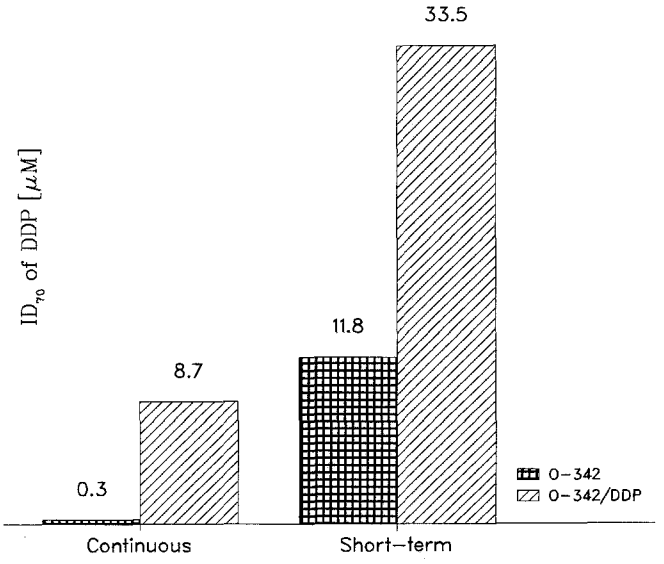

Fig. 4. Clonogenic assay: ID_{70} values for tumors O-342 and O-342/DDP after continuous and short-term exposure to DDP (Extrapolation from Tables 2 and 3)

reduced colony formation at 1 μM to 16% and 12% of control values, respectively. ADP and DAP were somewhat less effective in this setup, although they showed significant inhibition of colony formation at 1 and 10 μM, respectively (Table 2). In O-342/DDP, only the platinum compounds DDP and CTDP inhibited colony growth by >70% at concentrations of 10 μM, which can, however, be considered to be toxic in vivo (Table 2).

After STE, the effects of DDP and CTDP were less marked at a concentration of 10 μM. At a concentration of 100 μM, DDP and CTDP remained significantly active in O-342 (Table 3); remarkably, 100 μM DDP completely inhibited colony growth in O-342/DDP, probably due to the extremely high concentration of drug (LD_{50} of DDP, 43 μmol/kg). Equimolar concentrations of DBT showed low activity during CE; a dose-dependent inhibition of colony growth was not observed (Table 2). Following STE of O-342 at 100 μM, DBT marginally failed to reach significant activity; in O-342/DDP, on the other hand, DBT reduced colony growth significantly (7% at 100 μM; Table 3). Treatment with ruthenium compounds showed no significant inhibition of colony growth in O-342 during CE. The most active ruthenium complex, ICR, reduced colony formation to 48% of control values at a dose of

10 μM. All ruthenium compounds showed better activity in O-342/DDP (CE), in which ICR remained the most effective compound (Table 2); it reduced colony formation to 18% of control values at a concentration of 10 μM, which was at least comparable with results obtained using DDP. After STE ICR failed to show significant activity (Table 3).

Discussion

The soft-agar colony assay is widely used for in vitro drug screening [11, 26]. Prediction values for in vitro-in vivo correlation have been reported to be 95% for resistance and 60% for sensitivity of a tumor to a new agent [27]. Plating efficiencies of both tumors investigated were in the range of data observed for other rodent tumors [11]. According to Alberts et al. [1], CE in vitro of human ovarian tumor cell lines to DDP does not increase the inhibition of colony formation as compared with STE; the same concentrations of DDP used for 1 h vs CE did not show any change in colony growth. Our results do not confirm this finding; to achieve the same colony-growth inhibition in O-342, we applied 11.8 μM DDP in the STE and 0.31 μM in the CE setup. The decreased ID_{70}: O-342/DDP to O-342 ratio for DDP after STE (ratio, 2.8) as compared with CE (ratio, 28) may reflect a kind of exhaustion of resistance mechanisms by the high drug concentrations in O-342/DDP during STE. During CE, the resistant line apparently has a much higher capacity to counteract DDP activity than does the sensitive one, resulting in a 10-fold increase in this ratio. This result also suggests that different responses to new compounds between resistant and sensitive tumor lines might be elaborated more convincingly using CE. Dose-response relationships were also more clear-cut after CE. In addition, comparison of previously obtained in vivo results [29] with the present in vitro data underlines that CE better reflects the in vivo situation.

Of the new metal complexes investigated in the present work, CTDP clearly was the most active compound in the DDP-sensitive ovarian tumor line. In the DDP-resistant line, only the ruthenium compound ICR equaled CTDP's activity. During CE, CTDP was equally, if not more, active than DDP in both lines. This is promising, since its LD_{50} is 110 mg/kg (123 μm/kg) in mice (personal communication, B. K. Keppler), whereas that of DDP in mice is 13 mg/kg (43 μm/kg) [10]. The decreased activity of this compound in the STE assay as compared with the CE setup may be due to the presence of two dicarboxylate ring structures, possibly resulting in a slower rate of hydrolysis to the active form of the drug as described for other dicarboxylate-platinum ring systems such as, for instance, *cis*-diammine-1,1-cyclobutane-dicarboxylate (CBDCA) [10], which showed a toxicity pattern different from that of DDP in clinical trials [7]. In the resistant line (CE), a dose of 10 μM CTDP was marginally superior to DDP; it might be expected that this superiority increases at equitoxic doses. ADP was originally designed for the treatment of osteogenic tumors and bone metastases. It combines diammine-platinum as an antineoplastic moiety and biphosphonates as osteotropic moieties and produces less nephrotoxicity than does DDP [24]. In our experiments (CE), ADP showed significant action against O-342 but not against O-342/DDP. The third DDP analogue tested was DAP. It also consists of an osteotropic and an antineoplastic part. The 1,2-diammine-cyclohexano-platinum(II) (DACH)-component of this drug has been reported to lack cross-resistance to DDP [6, 17]. However, in our highly resistant tumor model, it failed to yield significant growth inhibition.

Recently, organometallic titanium compounds have attracted interest due to their antitumor and toxicologic profiles. We investigated DBT, a bis-beta-diketonato metal complex. Representatives of this group were first described by Keller et al. [19]; they showed good activity in different transplanted tumors. DBT was selected from this group as the most promising compound and entered clinical trial in 1986. It gave better results in the treatment of experimental autochthonous adenocarcinomas of the large bowel than did 5-fluorouracil (5-FU) [5]. Other groups have reported a similar action for their organometallic titanocene compounds in transplanted colonic carcinomas [25]. This type of neoplasm does not usually respond to DDP treatment. In our experiments in ovarian tumors, DBT caused no significant reduction in colony growth in the CE model. This may have been due to its high susceptibility to hydrolysis [20]. STE to DBT, on the other hand, effected a significant reduction in the colony growth of O-342/DDP at a concentration of 100 μM, which could be applied experimentally in vivo since the i.v. LD_{50} in Sprague-Dawley rats is 80 mg/kg (174 μm/kg); maximum tolerated single doses in clinical phase I studies ranged from 14 to 21 mg/kg (30–46 μm/kg) [20]. Although DBT was not superior to DDP in either line, two aspects nevertheless warrant further interest in the clinical application of this compound, especially in ovarian tumors. First, DBT has a different toxicity pattern and a lower emetic potential as compared with DDP [20]. On the other hand, due to its high susceptibility to hydrolysis, i.p. instillation of DBT may be superior to its systemic administration in the treatment of ovarian tumors or peritoneal carcinosis.

The ruthenium complexes thus far investigated have not entered clinical trial, mainly due to their lack of activity in experimental tumor systems as compared with DDP [2]. Some compounds showed good activity but were insoluble in water. New ruthenium analogues with the general formulae HB (RuB_2Cl_4) and $(HB)_2(RuBCl_5)$ – B being a nitrogen heterocycle – combine good therapeutic activity as compared with DDP and good water solubility [23]. All ruthenium compounds investigated by us had the same general formula: $HB(RuB_2Cl_4)$. ICR showed the highest activity of all congeners in P388 leukemia. At present, it is the best characterised compound of both of the new groups mentioned. It is also highly active in other transplanted tumors such as the Walker 256 carcinosarcoma, Stockholm ascitic tumor, B16 melanoma, sarcoma 180 and autochthonous colorectal tumors of the rat [12]. In autochthonous adenocarcinomas of the large bowel, IndCR is as active as ICR, although its toxicity is considerably lower [4].

In our tumor models, ICR showed the best effect of all ruthenium complexes, being comparable to DDP in O-342/DDP during CE. The reduced antitumor activity of

ICR during STE may be attributable to the decrease in its activation during STE as compared with CE. ICR contains a Ru(III) ion; in vivo, Ru(III) ions are supposed to be reduced to Ru(II) ions, which are highly reactive [9]. Activation of "prodrugs" containing Ru(III) is expected to be favored in areas that are low in oxygen and high in reductants, such as the reducing, hypoxic environment prevalent in many tumors. This reduction is more likely to occur during prolonged exposure. The better activity of all ruthenium compounds in the resistant tumor line O-342/DDP during CE corresponds with an intracellular level of reduced glutathione (GSH), which is almost twice as high in the O-342/DDP line than in its sensitive counterpart [8]; the higher amount of reducing capacity in the resistant tumor cells may have facilitated the activation of ruthenium complexes. During STE, the activity of ICR at its highest concentration seemed to be more favorable in the sensitive than in the resistant tumor line. This finding, however, is most probably of minor significance, as ICR did not fulfill the criteria for drug activity ($\leqslant 30\%$ of control values) in the STE model; in addition, there were no clear-cut dose-response relationships, and a statistically significant difference (>2 SE) between the results in both tumor lines could not be demonstrated for this compound. Altogether, our results support the findings of other groups [23] that ruthenium complexes can overcome resistance to DDP.

In conclusion, new analogues containing metal ions other than Pt may be able to broaden the spectrum of antitumor metal-complexes: DDP-sensitive tumors may be successfully treated with new analogues that have a higher therapeutic index; some compounds have been proven to overcome DDP resistance. Further studies, with particular emphasis on human tumor xenografts grown in nude mice, should concentrate on CTDP and ICR, since both compounds showed the most promising effects in our in vitro model.

Acknowledgements. We thank Dr. Dr. B. K. Keppler, Institute of Inorganic Chemistry, University of Heidelberg, for kindly supplying the new platinum, titanium and ruthenium metal complexes. We are indebted to G. Bernhold and S. Heil for their skillful technical assistance. This work was part of the program of the EORTC Screening and Pharmacology Group.

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