Eur J Cancer Clin Oncol, Vol. 25, No. 2, pp. 293–299, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Carrier Mediated Action of Platinum Complexes on Estrogen Receptor Positive Tumors*

NORBERT KNEBEL, CLAUS-DIETER SCHILLER, MARTIN R. SCHNEIDER, HELMUT SCHÖNENBERGER and ERWIN VON ANGERER†

Institut für Pharmazie, Universität Regensburg, Universitätsstrasse 31, D-8400 Regensburg, F.R.G.

Abstract—*Three 1,2-diaminoethane-dichloro-platinum(II) complexes linked to 5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole by spacer groups of varying length were evaluated for cytostatic activity in estrogen receptor (ER) positive and negative tumor cells.* In vitro, *only the growth of ER positive MCF-7 mammary tumor cells was inhibited whereas hormone independent MDA-MB 231 cells did not respond.* In vivo, *a strong inhibitory effect was only observed in ER positive MXT mammary tumors of the mouse. The complex with a hexyl group as spacer reduced the tumor weight by 89% after 6 weeks of treatment. The R 3327 Dunning prostatic tumor of the rat, which also contains ER was inhibited, too. Generally, the effect in ER negative tumors was weak. These findings can be rationalized by the high binding affinities of the complexes for ER. By the mouse uterine weight test it was shown that the endocrine activity of the complexes is very low. Therefore, a mode of action different from that exerted by estrogens or antiestrogens has to be assumed.*

INTRODUCTION

ENDOCRINE THERAPY of hormone-dependent mammary tumors has proven to be a valuable alternative to chemotherapy with cytostatic agents because of the low toxicity associated with drugs like antiestrogens or aromatase inhibitors [1–3]. However, this treatment is limited by the fact that approx. 40% of the patients with estrogen receptor positive tumors do not respond to endocrine manipulation [4]. This lack of response might be due to some additional transformation that has occured in the malignant cell [5]. The presence of estrogen receptors in tumors which do not respond initiated our search for new compounds that bind to the receptor but exert their antitumor effect by a different mode of action. Substances with receptor affinity and which carry a cytotoxic group were thought to be good candidates for this purpose. The 1,2-diaminoethane-dichloro-platinum(II) group was chosen as the cytostatic function because the parent compound *cis*-platinum is a potent antineoplastic agent against some tumors, especially against testicular cancer [6], but has low activity against breast cancer [7]. Receptor affinity of platinum complexes might make it possible to overcome the resistance of mammary tumors to *cis*-platinum. Our rationale is based solely on the presence of estrogen receptors in the malignant cells but not on their function as transmitters of hormonal signals.

The receptor affinity of the platinum complexes which we have synthesized is due to the 5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole moiety. The same structure is found in zindoxifene, a drug which we have developed as antiestrogen [8, 9]. The nitrogen of the indole is linked to 1,2-diaminoethane-dichloro-platinum(II) by an alkyl chain of four to six methylene groups (Fig. 1). The relative binding affinities of the three complexes are 1.0, 1.3 and 6.5 respectively (17β-estradiol: RBA = 100) [10]. They are only slightly lower than those of the corresponding ligands. Both the complexes and their ligands were tested for cytostatic activity *in vitro* using hormone-sensitive MCF-7 and hormone-independent MDA-MB 231 human mammary tumor cells. *In vivo*, the antineoplastic effect was determined in a hormone-dependent and an independent form of the transplanted MXT mammary tumor of the mouse. In addition, the effect of one of the complexes on rats bearing hormone-dependent R3327 Dunning prostatic carcinomas was studied.

MATERIALS AND METHODS

Chemicals

All of the complexes and their ligands were synthesized as described previously [10]. [^{3}H]Thymidine (80 Ci/mmol) was obtained from New England Nuclear, Dreieich, F.R.G. Hormones and biochemicals were purchased from Sigma, München, F.R.G.

Accepted 1 September 1988.
*Supported by the Deutsche Forschungsgemeinschaft (SFB 234) and the M. Lackas-Stiftung.
†To whom correspondence should be addressed.

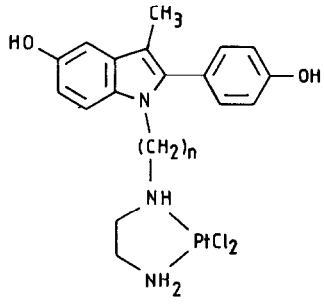

Fig. 1. Structural formulas of the platinum complexes 1-$PtCl_2$ (n = 4), 2-$PtCl_2$ (n = 5), *and* 3-$PtCl_2$ (n = 6).

MCF-7 human breast cancer cells

The MCF-7 cells (American Type Culture Collection) were grown in improved Minimal Essential Medium (MEM), as modified by Richter *et al.* [11] (Biochrom, Berlin, F.R.G.), supplemented with glutamine (0.3 g/l), gentamycin (60 mg/l) and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (CCS). CCS was prepared by incubation of 500 ml NCS with a dextran-coated charcoal pellet [12] for 4 h in a shaker at 0–4°C. The procedure was repeated with a fresh pellet. After each incubation, the charcoal was removed by centrifugation. The serum was sterilized through a 0.20 μm filter (Sartorius, Göttingen, F.R.G.) and stored at −20°C. All of the experiments were performed in the presence of phenol red except where noted.

Cells were grown in a humified incubator in 5% CO_2 at 37°C. Two weeks before the start of the experiment, cells were switched from NCS to CCS and received two additional media changes before they were harvested with 0.05% trypsin–0.02% EDTA in 0.15 M NaCl. They were syringed gently to prevent clumping, and approx. 2×10^4 cells in 2 ml were plated as replicates in six-well dishes (Falcon). Two days later, cells were switched to a medium containing the substances and 0.1% DMF in which the compounds had been dissolved. The medium of control wells contained an equal volume of DMF. On the 4th day, media were changed and substances added again. Three days later, cells were labeled with 1 μCi [^{3}H]thymidine per well for 2 h. Cells were washed with cold PBS and harvested in PBS containing 0.02% EDTA. After centrifugation, the cell pellet was resuspended in 1 ml of PBS and divided into two equal parts. One part was counted in a Z I Coulter Counter, the other one was sonicated. After addition of 4 ml of 10% trichloroacetic acid (TCA), the acid-insoluble fraction was collected on a 0.45 μm filter (Sartorius) and counted after addition of 10 ml scintillation liquid (Quicksz-int 212, Zinsser) in a Beckman LS 1801 scintillation counter.

MDA-MB 231 human breast cancer cells

The MDA-MB 231 cells (American Type Culture Collection) were grown in the same medium as described above, supplemented with 10% NCS and gentamycin (40 μg/ml). The experiments were performed as described for the MCF-7 cells with one exception: the incubation period was reduced from 5 to 2 days.

Transplanted MXT mammary tumors of the mouse

The MXT mammary tumors were generously provided by Dr A.E. Bogden, EG & G Mason Research Institute, Worcester, MA, U.S.A. and Dr G. Leclercq, Institute Jules Bordet, Brussels, Belgium. Hormone-sensitive tumors grew for 4–5 weeks in the host animals before transplantation. Tumor pieces of 1 mm^3 were serially transplanted into 8- to 9-week-old female B6D2F1 mice, obtained from Charles-River-Wiga (Sulzfeld, F.R.G.). Animals were assigned randomly in groups of 10 and treatment was started 24 h after transplantation. Drugs were dissolved or suspended in polyethylene glycol 400/0.9% saline (1:1) and administered subcutaneously on Monday, Wednesday and Friday. After a 6-week period of treatment, animals were killed and autopsied. Tumors were removed and weighed. The uterine dry weight was determined as described above. The change of body weight between start and end of therapy was recorded in order to detect obvious toxicity.

Hormone-resistant tumors were kept in ovariectomized B2D2F1 mice. Treatment was started 24 h after transplantation and lasted 2 weeks. The administration scheme was the same one as outlined for hormone-sensitive tumors. Since the hormone-sensitive tumors cannot be dissected free of connective tissue, the tumor area was determined instead of tumor weight. The tumor area was obtained by transdermal caliper measurements of two perpendicular axes, one across the largest diameter.

Immature mice uterine weight tests

Immature female mice (20 days old, of the NMRI strain) from Ivanovas (Kisslegg, F.R.G.) were randomly divided into groups of six to 10 animals. To determine estrogenic activity, compounds were dissolved in polyethylene glycol/0.9% saline (1:1, 100 μl/animal) and injected subcutaneously on 3 consecutive days. Control animals received the vehicle alone. Twenty-four hours after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid–40% formaldehyde–glacial acetic acid, 15:15:1 by vol.) for 2 h. Uteri were freed from connective tissue, washed with a saturated alcoholic solution of LiCl, dried at 100°C for 24 h and weighed. Relative uterine weight was calculated by

the formula: uterine dry weight (mg)/body weight (g), multiplied by 100.

To determine the antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the complexes or the ligands.

R3327 Dunning prostatic carcinoma of the rat

Male Copenhagen × Fisher F_1 rats bearing hormone-dependent R3327 Dunning rat adenocarcinoma were kindly provided by Dr. N. Altman, Papanicolaou Cancer Research Institute, Miami, U.S.A. [13]. Ten weeks after tumor implantation, they were randomly distributed into groups of seven to eight rats. In one group, rats were injected with the platinum complex dissolved in polyethylene glycol/0.9% saline (1:1) once weekly, followed by 1 week's interval. In the second group, animals were castrated on day 1 of treatment; animals of the control group received only the vehicle. Tumor area was determined weekly by calculating the product of caliper measurements made in two perpendicular diameters. At the end of therapy, tumors, seminal vesicles, testicles and prostates were removed and dissected free from adhering tissue and fat. Tumors, prostates and testicles were blotted dry and weighed; seminal vesicles were dried overnight (100°C) before weighing.

RESULTS

Effect on human mammary tumor cells in vitro

The cytostatic activity of the platinum complexes was evaluated *in vitro* using both a hormone sensitive and a hormone independent human mammary tumor cell line. With MDA-MB 231 cells, which can be considered as estrogen receptor negative, no cytostatic action of the complexes was observed up to a concentration of 10^{-5} molar. At that concentration, 2-$PtCl_2$ and 3-$PtCl_2$ complexes showed a marked inhibitory effect on cell number and thymidine incorporation of estrogen receptor positive MCF-7 cells (Table 1). The highest activity was found with 2-$PtCl_2$ which possesses a C-5 spacer group. Interestingly, ligands 2 and 3 showed similar activity in estrogen sensitive mammary tumor cells. We assume that this effect is mainly due to the presence of the basic diamino side chain. It has been shown in the triphenyl ethylene series that the aminoalkyl side chain gives rise to a strong growth inhibiting effect on MCF-7 cells [14].

Most of the experiments with these cells were performed both in the presence and in the absence of phenol red in the medium, because it has been reported that the estrogenic effect of this dye stimulates cell proliferation and is a prerequisite for the

Table 1. *Effect of the platinum complexes* 1-$PtCl_2$–3-$PtCl_2$ *and ligands* 2 *and* 3 *on the growth of MCF-7 human mammary tumor cells*

Compound	Concentration (M)	Cell No./dish* × 10^4	Percentage T/C	[^{3}H]Thymidine incorporation cpm × 10^3	Percentage T/C
Control		91.6 ± 9.7		22.2 ± 1.0	
1-$PtCl_2$	1×10^{-5}	78.5 ± 4.6	85‡	14.2 ± 1.0	64‡
	1×10^{-6}	92.8 ± 8.0	101	17.6 ± 1.8	80‡
Control		50.4 ± 5.5		30.6 ± 1.8	
2-$PtCl_2$	1×10^{-5}	5.5 ± 0.8	11‡	0.4 ± 0.02	1‡
	5×10^{-6}	12.2 ± 0.8	24‡	3.2 ± 0.8	11‡
	1×10^{-6}	28.1 ± 1.3	56‡	17.6 ± 2.0	58‡
Control		35.5 ± 2.5		43.2 ± 2.6	
2-$PtCl_2$	1×10^{-6}	23.1 ± 1.0	65‡	21.0 ± 4.4	48‡
	5×10^{-7}	28.1 ± 0.4	79	34.2 ± 2.6	79
	1×10^{-7}	34.8 ± 2.1	98	47.4 ± 2.2	110
2	1×10^{-6}	7.6 ± 0.2	21‡	15.4 ± 3.4	35‡
	5×10^{-7}	11.2 ± 0.9	31‡	28.0 ± 2.8	65‡
	1×10^{-7}	26.7 ± 0.7	75‡	30.4 ± 6.4	70‡
Control		65.9 ± 6.7		25.2 ± 0.4	
3-$PtCl_2$	1×10^{-5}	37.3 ± 4.2	57‡	7.8 ± 0.4	31‡
	5×10^{-6}	57.1 ± 4.2	87‡	11.0 ± 0.4	44‡
	1×10^{-6}	63.3 ± 8.4	96	13.9 ± 0.4	55‡
3	1×10^{-5}	23.1 ± 2.1	35‡	2.8 ± 0.6	11‡
	5×10^{-6}	27.9 ± 2.9	42‡	2.8 ± 0.4	11‡
	1×10^{-6}	39.8 ± 4.2	60‡	8.5 ± 0.2	39‡

*Cell No. based on Coulter counts on day 7, mean of six dishes ± S.D.
†Radioactivity/dish; mean of six dishes ± S.D.
‡Significant inhibition ($P < 0.01$).

detection of an antiestrogenic effect in this assay [15]. We did not find a marked and consistent difference in our results using either phenol red or not. In this respect, the cells we used are similar to those grown for a longer period of time in the absence of estrogens [16].

Effects on transplanted MXT mammary tumors of the mouse

The *in vivo* antitumor activity was determined in two different forms of the transplanted MXT mammary tumor of the BDF-1 mouse. One tumor (MXT-M3.2 [17]) contains estrogen receptors and responds to endocrine manipulation, whereas the MXT-ovex tumor [18], which is propagated in ovariectomized mice, can be considered hormone-independent. The ligands showed some activity in this model, preferentially in hormone sensitive tumors, but the results were not consistent (Fig. 2). A marked difference between these two tumor models was observed with the platinum complexes 2-$PtCl_2$ and 3-$PtCl_2$. They exhibited a strong inhibitory effect only on the growth of estrogen receptor positive tumors but low activity in receptor negative tumors (Fig. 3). At a dose of 3 × 20 mg/kg body weight/week, the maximum effect on tumor weight was 11% T/C after 6 weeks of treatment (3-$PtCl_2$). In all of the groups, the mean body weight of the animals increased by 10–20% as in the control group. No stimulation of uterine growth due to an estrogenic action was observed. The 1-$PtCl_2$ complex showed no antitumor activity in this *in vivo* model.

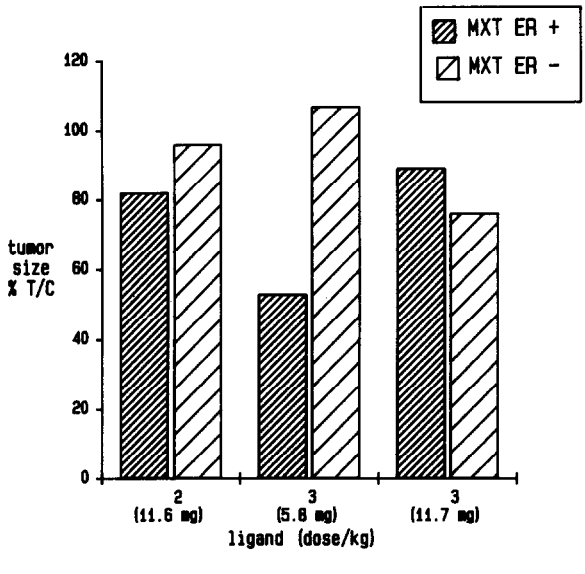

Fig. 2. Effect of diamino ligands 2 and 3 on the growth of transplanted estrogen receptor positive (ER+) and negative (ER−) MXT mammary tumors of the BDF-1 mouse. Animals received the doses listed three times/week subcutaneously. After 6 weeks of treatment, ER+ tumors were removed and weighed. The size of ER− tumors was estimated by transdermal caliper measurements 2 weeks after the start of treatment. Mean tumor weights and mean tumor areas respectively were used for the calculation of T/C values.

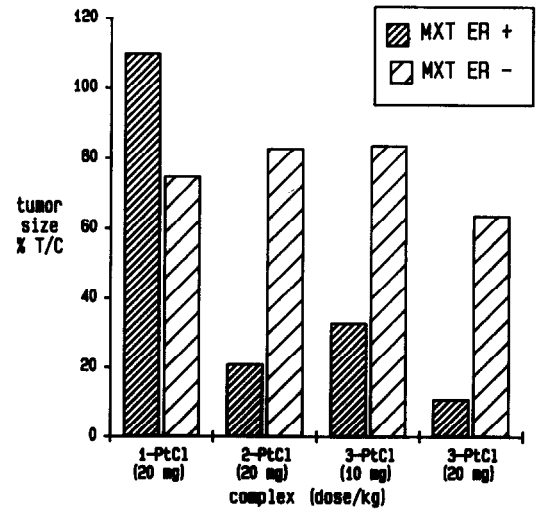

Fig. 3. Effect of the platinum complexes 1-$PtCl_2$–3-$PtCl_2$ *on the growth of transplanted estrogen receptor positive (ER+) and negative (ER−) MXT mammary tumors of the BDF-1 mouse. For details see Fig. 2.*

Endocrine activity

Since all of the complexes and their ligands display high binding affinities for the estrogen receptor, an endocrine activity such as an estrogenic or antiestrogenic effect could be expected. Therefore, all of the compounds were tested for their uterotrophic and antiuterotrophic activity in mice. In this assay, immature mice received various doses of the drug, and the increase in uterine dry weight was measured thereafter [19]. Antagonistic activity was determined by simultaneous administration of the test compound and estrone (0.4 μg) and calculation of the inhibition of estrone-stimulated uterine growth. Despite the high binding affinities of the compounds tested, no marked estrogenic effect was observed in the mouse uterine weight test (Table 2). The growth stimulation was less than 20% of that exerted by 0.4 μg of estrone, except for the 2-$PtCl_2$ complex at the highest dose (32%). From these data we deduced that both the complexes and their ligands possess no or only low hormonal activity at the doses applied.

Effect on rats bearing R3327 Dunning prostatic carcinomas

Since carcinomas of the prostate are known to contain estrogen receptors besides androgen receptors [20], they are a potential target for platinum complexes with binding affinity for the estrogen receptor. In an experiment with established hormone-dependent R3327H Dunning prostatic carcinomas in rats, we observed a strong inhibition of tumor growth following the application of 50 mg 3-$PtCl_2$/kg once weekly (Fig. 4). However the effect

Table 2. Estrogenic and antiestrogenic activity of platinum complexes 1-$PtCl_2$–3-$PtCl_2$ *and their ligands* 1–3 *in the mouse uterine weight test*

Compound	Dose* (μg)	Uterotrophic test Relative uterine weight†	Antiuterotrophic test Relative uterine weight†‡	Inhibition (%)
Control		12.1 ± 3.0		
1	14	13.9 ± 3.1	45.9 ± 9.2	6
	71	13.2 ± 2.6	46.4 ± 9.5	4
	142	14.5 ± 2.4	42.8 ± 7.7	15
Estrone	0.4	48.0 ± 3.0		
1-$PtCl_2$	25	12.7 ± 3.9	46.4 ± 6.2	4
	125	14.9 ± 6.2	48.1 ± 8.8	
	250	11.1 ± 4.2	41.2 ± 10.2	19
Control		16.0 ± 4.3		
2	15	15.5 ± 2.0	53.5 ± 9.8	
	73	20.1 ± 7.1	47.0 ± 10.3	
	145	18.2 ± 4.4	60.1 ± 9.7	
Estrone	0.4	45.4 ± 3.1		
2-$PtCl_2$	25	18.5 ± 3.9	56.8 ± 5.2	
	125	20.7 ± 4.5	44.6 ± 9.5	3
	250	25.3 ± 0.3	54.1 ± 9.6	
Control		13.5 ± 1.8		
3	14	12.1 ± 2.5	44.0 ± 6.8	10
	73	14.1 ± 2.9	44.9 ± 9.4	8
	147	14.3 ± 3.6	42.8 ± 4.0	14§
Estrone	0.4	47.6 ± 8.4		
3-$PtCl_2$	25	13.8 ± 4.4	45.1 ± 7.4	7
	125	16.9 ± 2.8	46.7 ± 9.2	3
	250	18.3 ± 3.9	53.4 ± 6.8	

*Dose per animal, administered on 3 consecutive days s.c.
†Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 6–10 animals ± S.D.
‡Simultaneous administration of 0.4 μg of estrone/animal and day.
§Significant ($P < 0.05$).

of castration was not reached. At the end of therapy, the weights of prostates, testicles and seminal vesicles were determined in order to evaluate the endocrine effects of the different treatment modalities (Table 3). No significant difference was observed between animals treated with platinum complex and the control group. Therefore, an antigonadotrophic effect as observed for the structurally related antiestrogen zindoxifene [21] or some of the diphenylethylene platinum complexes [22] can be ruled out.

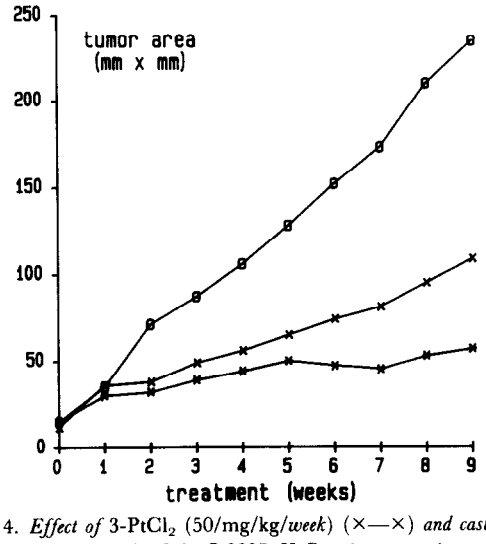

Fig. 4. Effect of 3-$PtCl_2$ (50/mg/kg/*week*) (×—×) *and castration* (*—*) *on the growth of the R* 3327 *H Dunning prostatic carcinoma transplanted into rats (control:* o—o).

DISCUSSION

The aim of these investigations was the development of drugs with a specific cytostatic action on estrogen receptor positive tumors with a mechanism different from those exerted by estrogens or antiestrogens. The platinum complexes used in this study are very appropriate for this purpose because they possess high binding affinities for the estrogen receptor but are devoid of hormonal activity as shown by the uterine weight test. The diaminoethane-dichloro-platinum group that should serve as effective group has lost its unspecific cytostatic properties when linked to the 2-phenylindole. Therefore, estrogen receptors negative MDA-MB 231 mammary tumor cells and hormone-independent MXT tumors did not respond to treatment with the complexes. These observations were confirmed by

Table 3. Effect of 3-$PtCl_2$ (50 mg/kg/*week*) *on prostate, testicle, seminal vesicle and body weight of rats bearing R* 3327 *Dunning prostate carcinomas after* 9 *weeks of treatment*

	Mean prostate weight (mg)	Mean testicle weight (mg)	Mean seminal vesicle weight (mg)	Change of mean body weight (%)
Control	291	2832	202	+9
Castrated	46	—	22	+4
3-$PtCl_2$	224	2547	164	−1

results of experiments performed with mice bearing P388 leukemia. No increase of lifespan was reached (data not shown). Contrary to these findings, two out of the three complexes tested strongly inhibited the growth of estrogen receptor positive MCF-7 breast cancer cells *in vitro*. The *in vivo* experiments with transplanted MXT mouse mammary tumors led to the same result: only receptor positive tumors responded to therapy with 2-$PtCl_2$ and 3-$PtCl_2$. *In vivo*, the ligands were inactive as well. These results strongly support the assumption of a specific action of these cytostatics mediated by the estrogen receptor.

Since the *in vivo* activities of the platinum complexes do not differ very much from those obtained *in vitro*, we assume that the unchanged drug or the same metabolite is effective at the target. Studies with *cis*-platinum and other platinum complexes have shown that the chloride ions can be replaced by hydroxo groups or water under physiological conditions, whereas the platinum–amino bond is not likely to break, unless the complex is fixed to a target molecule [23]. Therefore, the active molecular species is probably the unchanged 1,2-diaminoethane-dichloro-platinum(II) complex with chloride, hydroxide or water as leaving group.

The specific activity of the platinum complexes studied raises questions concerning the mode of action on a cellular level. From our results, obtained with immature mice, we exclude an endocrine antitumor effect as observed with estrogens or antiestrogens. We considered a possible inactivation of the estrogen receptor as well, since it is known that the chloro ligands of the platinum can be displaced by other nucleophiles like thiol groups of proteins [24]. We found by exchange experiments with [^{3}H]estradiol that the binding of the complexes to the receptor is completely reversible (data not shown). If the complex reacts with the DNA after dissociation from the receptor one would expect similar results both in receptor positive and negative cells. Since this is not the case, the antitumor effect must be mediated by the estrogen receptor in an unknown manner. A specific accumulation of the complexes in target cells has to be discussed as well. Further experiments will be performed after we have established an assay for these platinum complexes.

For evaluation of these specifically acting complexes, we compared their antineoplastic activity with the antitumor effect of zindoxifene that acts only by an endocrine mode and the effect of the cytostatic *cis*-platinum. Equimolar doses of zindoxifene showed the same growth inhibiting effect on hormone-dependent MXT tumors as the 3-$PtCl_2$ complex does [9]. *Cis*-platinum in a subtoxic dosage (3 × 1.4 mg/kg body weight/week) was slightly more active [22]. Since estrogen receptor positive human mammary carcinomas resistant to conventional endocrine therapy are not very likely to respond to zindoxifene or *cis*-platinum, the application of the platinum complexes with a carrier mediated mode of action might offer a new therapeutic alternative.

Acknowledgements—We thank M. Beer, R. Brunner-Ploss and F. Wiesenmayer for their technical assistance.

REFERENCES

1. Pearson OH, Manni A, Arafah BM. Anti-estrogen treatment of breast cancer. An overview. *Cancer Res* (Suppl.) 1982, **42**, 3424s–3429s.
2. Jordan VC, Fritz NF, Gottardis MM. Strategies for breast cancer therapy with antiestrogens. *J Steroid Biochem* 1987, **27**, 493–498.
3. Brodie AMH. Aromatase inhibition and its pharmacologic implications. *Biochem Pharmacol* 1985, **34**, 3213–3219.
4. Maass H, Jonat W, Stolzenbach G, Trams G. The problem of nonresponding estrogen receptor-positive patients with advanced breast cancer. *Cancer* 1980, **46**, 2783–2787.
5. Kasid A, Lippman ME. Estrogen and oncogene mediated growth regulation of human breast cancer cells. *J Steroid Biochem* 1987, **27**, 465–470.
6. Loehrer PJ, Einhorn LH. Cisplatin. *Ann Intern Med* 1984, **100**, 704–713.
7. Arteaga CL, Forseth BJ, Clark GM, Von Hoff DD. A radiometric method for evaluation of chemotherapy sensitivity: results of screening a panel of human breast cancer cell lines. *Cancer Res* 1987, **47**, 6248–6253.

8. von Angerer E, Prekajac J, Strohmeier J. 2-Phenylindoles. Relationship between structure, estrogen receptor affinity, and mammary tumor inhibiting activity in the rat. *J Med Chem* 1984, **27**, 1439–1447.
9. von Angerer E, Prekajac J, Berger MR. The inhibitory effect of 5-acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) on estrogen-dependent mammary tumors. *Eur J Cancer Clin Oncol* 1985, **21**, 531–537.
10. Knebel N, von Angerer E. Platinum complexes with binding affinity for the estrogen receptor. *J Med Chem* 1988, **31**, 1675–1679.
11. Richter A, Sandford KK, Evans VJ. Influence of oxygen and culture medium on plating efficiency in some mammalian tissue culture cells. *JNCI* 1972, **49**, 1705–1712.
12. Scholl SM, Huff KK, Lippman ME. Anti-estrogenic effects of LY 117018 in MCF-7 cells. *Endocrinology* 1983, **113**, 611–617.
13. Smolev JK, Heston WDW, Scott WW, Coffey DS. Characterization of the Dunning R 3327 H prostatic adenocarcinoma: an appropriate animal model for prostatic cancer. *Cancer Treat Rep* 1977, **61**, 273–287.
14. Paridaens RJ, Leclercq G, Heuson JC. Steroid hormones, analogs and antagonists. In: Pinedo HM, Chabner BA, eds. *Cancer Chemotherapy*. Amsterdam, Elsevier, 1983, 171–192.
15. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci USA* 1986, **83**, 2496–2500.
16. Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y. Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. *Cancer Res* 1987, **47**, 4355–4360.
17. Schneider MR, Ball H, Schönenberger H. Acetoxy-substituted 1,1,2-triphenylbut-1-enes with antiestrogenic and mammary tumor inhibiting properties. *J Med Chem* 1985, **28**, 1880–1885.
18. Schuderer ML, Schneider MR. Cytotoxic esters of 1,1-bis-(4-hydroxyphenyl)-2-phenylbut-1-ene with selective antitumor activity against estrogen receptor-containing mammary tumors. *J Cancer Res Clin Oncol* 1987, **113**, 230–234.
19. Rubin BL, Dorfman AS, Black L, Dorfman RI. Bioassay of estrogens using the mouse uterine response. *Endocrinology* 1951, **49**, 429–439.
20. Ip MM, Milholland RJ, Rosen F. Functionality of estrogen receptor and tamoxifen treatment of R 3327 Dunning rat prostate adenocarcinoma. *Cancer Res* 1980, **40**, 2188–2193.
21. Schneider MR, von Angerer E, Höhn W, Sinowatz F. Antitumor activity of antiestrogenic phenylindoles on experimental prostate tumors. *Eur J Cancer Clin Oncol* 1987, **23**, 1005–1015.
22. Karl J, Gust R, Spruss T *et al.* Ring-substituted [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes: compounds with a selective effect on hormone-dependent mammary carcinoma. *J Med Chem* 1988, **31**, 72–83.
23. Lippert B, Schöllhorn H, Thewalt U. Facile substitution of NH3 ligands in a diplatinum(III) complex of 1-methyluracil. *Inorg Chem* 1986, **25**, 407–408.
24. Kraker A, Schmidt J, Krezoski S, Petering DH. Binding of *cis*-dichlorodiammine platinum(II) to metallothioneinin Ehrlich cells. *Biochem Biophys Res Commun* 1985, **130**, 786–792.