Tumor Inhibiting [1,2-Bis(fluorophenyl)ethylenediamine]platinum(II) Complexes*)

Part IV: Biological Evaluation - *in vivo* Studies on the P 388 Leukemia

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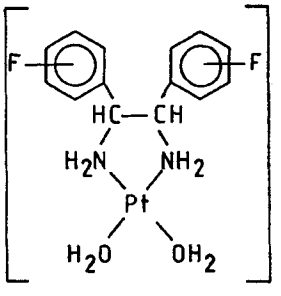
In experiments on the P 388 leukemia of the mouse diaqua-[1,2-bis-(fluorophenyl)ethylenediamine]platinum(II) sulfates and nitrates show a strong antitumor activity irrespective of the position of the fluorine atom (ortho, meta or para) and of their configuration (R,R/S,S or R,S).

Tumorhemmende [1,2-Bis(fluorphenyl)ethylendiamin]platin(II) Komplexe, 4. Teil: Biologische Prüfung - *In vivo* Studien an der P 388 Leukämie

In Versuchen an der P 388 Leukämie der Maus zeigten Diaqua-[1,2-bis(fluorphenyl)ethylendiamin]platin(II)sulfate und -nitrate eine starke Antitumorwirkung unabhängig von der Stellung des Fluoratoms (ortho, meta oder para) und ihrer Konfiguration (R,R/S,S oder R,S).



Compd.	Config.	F-Position	Counter Ion	Abbreviation
25	D,L	2	SO_4	D,L-2F-PtSO_4
26	D,L	3	SO_4	D,L-3F-PtSO_4
27	D,L	4	SO_4	D,L-4F-PtSO_4
28	Meso	2	SO_4	Meso-2F-PtSO_4
29	Meso	3	SO_4	Meso-3F-PtSO_4
30	Meso	4	SO_4	Meso-4F-PtSO_4
31	D,L	4	NO_3	D,L-4F-Pt$(NO_3)_2$
32	Meso	4	NO_3	Meso-4F-Pt$(NO_3)_2$

In experiments on the P 388 D_1 leukemia cell line we demonstrated that [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes of the same configuration (R,R/S,S and R,S, respectively) are comparably active irrespective of the position of the fluorine atom (ortho, meta or para) and the nature of the "leaving group" (Cl^- or H_2O)[1]. However, the R,R/S,S configurated compounds proved to be markedly more active than those of the R,S series and comparable to cisplatin[1]. In addition, some of these compounds, the diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfates and nitrates, showed a strong inhibitory effect on the hormone dependent MXT - mammary carcinoma of the mouse[2].

In this publication we report on the evaluation of the water soluble diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates (**25** to **30**) and nitrates (**31** and **32**) on the lymphocytic P 388 leukemia of the mouse.

Results

This tumor was induced by topical administration of methylcholanthrene on the skin of DBA/2 mice and established as ascites tumor[3]. In 1975 the lymphocytic P 388 leukemia of the mouse was introduced as prescreen by

Table 1: Influence of the Number of Transplanted P 388 Leukemia Cells on the Body Weight and the Median Survival Time of CD_2F_1 Mice[6]

cell number x 10^5	body weight ± s(g) day 1	day 5	day 9	median day of survival (range)
0,6	21,3 ± 1,5	20,8 ± 1,4	24,9 ± 2,5	10,5 (10 - 12)
0,9	20,2 ± 2,5	20,6 ± 2,6	24,3 ± 2,6	10,0 (10 - 14)
9,0	20,2 ± 1,2	21,7 ± 1,5	24,9 ± 1,9	9,0 (8 - 11)

*) Dedicated to Professor Dr. *H. Heimpel*, Head, Department of Medicine III and Chairman, Tumor Center Ulm, University of Ulm, on the occasion of his 60th birthday.

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Table 2: Activity of Cisplatin on the P 388 Leukemia of the Mouse Depending on the Number of Passages[6)]
a) S: solvent = polyethylene glycol 400/1,8 % NaCl solution 1:1. Cisplatin: dosage: 1,5 mg/kg on day 1, 5 and 9 b) Passages: number of transplantations which were used in the preservation of the P 388 cell line c) Mean difference in body weights between day 5 and day 1.[d] T: therapy; C: control e) Ratio of median survival time of treated and untreated leukemic mice in percent

compd.[a]	passage[b] (No)	body weight (g)[c] day 5 - day 1	T - C[d] (g)	median day of survival (range)	% T/C[e]
S	2	1,9	-	9,0 (8 - 13)	-
Cisplatin	2	- 0,7	2,6	21,0 (14 - 25)	233
S	14	2,5	-	10,0 (8 - 13)	-
Cisplatin	14	- 3,3	5,8	25,0 (20 - 26)	250
S	26	1,2	-	10,5 (9 - 13)	-
Cisplatin	26	- 2,0	3,2	20,5 (18 - 23)	195
S	36	1,5	-	11,0 (8 - 13)	-
Cisplatin	36	- 2,2	3,7	22,5 (17 - 26)	205
S	48	1,0	-	10,0 (9 - 13)	-
Cisplatin	48	- 2,1	3,1	21,0 (9 - 26)	210

the National Cancer Institute - USA (NCI)[4)]. In the therapeutic experiment, about 10^6 leukemia cells are transplanted intraperitoneally into 8 weeks old female CD_2F_1 mice (day 0). Test substances, too, are administered intraperitoneally on days 1, 5, and 9. The activity of a drug is evaluated by registration of the median day of survival in comparison to the solvent treated control. In accordance with the NCI protocol[5)], drugs showing a % T/C value ≥ 125 are considered antitumor active.

The influence of the number of transplanted leukemia cells on the median survival time of the test animals is relatively low (Table 1). In the range of 0.6 to 9.0 x 10^5 cells per mouse we could not detect an acceleration of the increase of animal weights (caused by an increase of tumor volume). Only at the highest cell number (9 x 10^5) a small decrease in the survival time was seen.

The experiment on the P 388 leukemia of the mouse gives also hints on the acute toxicity of the test compound, if the change of body weights is registered in the course of the trial (day 5 - day 1). A drug which produces a body weight reduction > 4 g is considered toxic.

Up to the 50th in vivo-passage the P 388 leukemia does not show alterations in morphology and growth rate. Under these conditions (*in vivo*-passages ≤ 50) median survival times of mice inoculated with P 388 cells were comparable (cf. Table 2). Consistently, the therapeutic effect of cisplatin was not influenced by the number of *in vivo*-passages of the P 388 cells (Table 2). Only after >> 50 *in vivo*-passages the morphology of the cells was changed into a reticular cell like phenotype, the growth rate was diminished and the survival time of untreated animals was elongated. This second tumor cell type was stronger inhibited by cisplatin[6)]. Therefore, to ensure the comparability of test data, we used only P 388 cells from *in vivo*-passages ≤ 50.

For the evaluation of the antitumor activity of [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes on the P 388 leukemia of the mouse the dose range 1, 2, and 4 x 10^{-5} mol/kg was selected as to correspond to the values found for 50% inhibition (IC_{50}) of the P 388 D_1 cell line (compare Table 1 in ref. 1) and to the highest tolerated single dose (about 4 x 10^{-5} mol/kg). The dose of 4 x 10^{-5} mol/kg amounts to about the 10-fold of the IC_{50} values of the R,S configurated complexes and to the 100-fold of those of the R,R/S,S configurated analogues. In accordance with our results on the P 388 D_1 leukemia cell line the diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates **25** to **30** showed a comparably strong and dose dependent in vivo antitumor activity in the R,R/S,S series **25** to **27** as well as in the R,S series **28** to **30**, regardless of the fluorine position (compare Table 3 with Table 1 in ref. 1). However, in contrast to our *in vitro* results the related diastereomers are similarly active. As expected, the diaqua[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) sulfates **27** and **30** and nitrates **31** and **32** do not differ in their antitumor effect (Table 3).

The acute toxicity, evaluated from the weight difference day 5 - day 1, was more pronounced in the R,R/S,S series than in the R,S series (Table 3). This required a reduction of the number of injections to 2 in the leukemia mouse test with compounds **25** (D,L-2F-$PtSO_4$) and 26 (D,L-3F-$PtSO_4$) at higher dosage range. The 2-position of the fluorine atom seems to promote toxic side effects. Compound **25** (D,L-2F-$PtSO_4$) proved to be much more toxic than all other test compounds. After administration of 4 x 10^{-5} mol/kg **25** (day 1), 4 out of 6 animals died on day 4 and 2 on day 8 (Table 3). However, by transformation of **25** into its dichloroplatinum(II) derivative (**13** in Table 3), a strong reduction of acute toxicity could be achieved (compare also the comments in part "discussion").

Discussion

On the basis of results of a colony forming assay (TCA) using the P 388 D_1 leukemia cell line as test model, one could assume that in the dosage of 4 x 10^{-5} mol/kg an elimination of the tumor cells and thereby a cure is achievable with R,R/S,S configurated compounds but not with their R,S-configurated counterparts (compare Table 2 in ref. 1)[7)]. In contrast to our expectation, the R,R/S,S configurated diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates **25** to **27** and nitrate **31** caused no cures in the mouse leukemia experiment, even at subtoxic dosage.

The discrepancy between the results obtained *in vitro* (TCA) and *in vivo* with R,R/S,S configurated diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) salts **25** to **27**, **31** and **32** can be explained by a poor bioavailability

Table 3: Antitumor Activity of Diastereomeric Diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) Sulfates and Nitrates on P 388 Leukemia of the CD_2F_1 Mouse

[a] Ip-administration on day 1, 5, and 9 [b] Mean difference in body weights between day 5 and day 1 [c] Ratio of median survival time of treated and untreated leukemic mice in percent [d,e] Values belong to controls d and e [f] Control: median survival time 9 days [g] Control: median survival time 10 days [h] **13**: [1,2-Bis(2-fluorophenyl)ethylenediamine]dichloroplatinum(II) [1)] On day 5 no injection [2)] On day 9 no injection [3)] Single administration on day 1; 4 animals died on day 4 and 2 animals on day 8

compound	single dose[a] x 10^{-5} [mol/kg]	median day of survival (range)		animal weight change d5-d1[b] [g]	% T/C[c]
D,L-2F-$PtSO_4$ [e]	1	18	(14-20)	-1.0	200
(25)	2 [2)]	20	(14-22)	-3.2	222
	4 [3)]	-	-	-	-
D,L-3F-$PtSO_4$ [e]	1	17	(16-17)	0.4	189
(26)	2	17.5	(16-19)	-1.3	194
	4 [1)]	18	(16-19)	-4.0	200
D,L-4F-$PtSO_4$ [f]	1	14	(6-16)	-1.9	156
(27)	2	14.5	(13-18)	-0.1	161
	4	18	(18)	-1.2	200
meso-2F-$PtSO_4$ [e]	1	17	(9-19)	0.7	189
(28)	2	23	(20-25)	-2.6	256
	4 [1)]	21	(12-22)	-2.9	233
meso-3F-$PtSO_4$ [e]	1	17	(16-19)	0.4	189
(29)	2	17	(15-19)	0.0	189
	4	22.5	(19-24)	-1.5	250
meso-4F-$PtSO_4$ [d]	1	15.5	(14-16)	1.9	172
(30)	2	17	(5-19)	0.8	188
	4	22	(18-23)	-0.9	244
D,L-4F-$Pt(NO_3)_2$ [d]	1	17	(16-18)	1.5	188
(31)	2	19	(17-23)	-1.9	211
	4	21	(19-22)	-4.1	233
meso-4F-$Pt(NO_3)_2$ [d]	1	17	(14-19)	1.5	188
(32)	2	18.5	(17-21)	0.2	206
	4	25	(5-26)	-1.9	278
D,L-2F-$PtCl_2$ [g,h]	2.4	19	(17-21)	0.9	181
(13)	4.9	20.5	(18-22)	-1.7	195
	9.8	17	(11-18)	-2.9	162
Control [d]	-	9	(6-13)	1.6	-
Control [e]	-	9	(8-12)	1.4	-

under *in vivo* conditions. A prerequisite for a curative cancer chemotherapy is a drug level sufficiently high and persistent to cause an eradication of tumor cells which possess an unlimited capacity of proliferation (tumor stem cells). Of these two requirements, the achievement of a cytotoxic drug level is certainly ensured under the used experimental conditions (intraperitoneal administration). However, the P 388 ascites tumor seems not to be exposed to a cytotoxic drug concentration for a period long enough to eradicate the tumor stem cells. Previous cell culture experiments with compounds **27** (D,L-4F-$PtSO_4$) and **31** (D,L-4F-$Pt(NO_3)_2$) showed that indeed long incubation times (6 h for a % T/C $\geq$ 90[1)]) are necessary for total inhibition. To understand these correlations pharmacokinetic studies are required, especially those on changes of the drug level with time after ip administration.

Persumably, the irreversible binding to bionucleophils, mainly plasma proteins, plays a major role in the reduction

of bioavailability of the R,R/S,S configurated complexes. The inactivation process can be delayed by substitution of the highly reactive "leaving group" H_2O by less reactive groups like Cl^- as well as by change in ligand configuration from R,R/S,S to R,S.

Platinum(II) complexes with more stable bound "leaving groups" like Cl^- are weakly reactive prodrugs. They are slowly hydrolyzed into the active diaquaplatinum(II) species, which are responsible for the reaction with DNA. This process is favored as mode of action of platinum(II) complexes (compare ref. 1). The inactivation of platinum complexes of the prodrug type is impeded during the transport to the tumor cell.

The alternative opportunity to delay the inactivation of diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) salts, the change of ligand configuration from R,R/S,S to R,S, has the advantage of a better water solubility than that of dichloroplatinum(II) derivatives. The lower tendency of the R,S-configurated complexes to react with bionucleophils is explained by a steric shielding of Pt by the axially standing phenyl residue. In the R,R/S,S configurated complexes, which are thought to exist in a conformation with equatorially oriented phenyl residues, the Pt is more easily accessible for a reaction with bionucleophils.

The lower reactivity of the two structure variants of the [1,2-bis-(fluorophenyl)ethylenediamine]platinum(II) complexes, the R,R/S,S configurated dichloroplatinum(II) derivatives and the R,S configurated diaquaplatinum(II) salts, needs of course a considerably longer interaction with the tumor cells to achieve comparable cytotoxic effects (compare fig. 1 and 2 in ref. 1). To obtain a curative cancer chemotherapy an adequate kinetic behavior of the platinum(II) complexes in the reaction with nucleophils is essential. Too fast or too slowly reacting complexes are weakly active, since they are either inactivated during their transport to the tumor cell or the necessary drug level cannot be maintained long enough.

In the case of the dichloroplatinum(II) type the kinetics of the $Cl\text{-}H_2O$-exchange (that is the transformation of the prodrug into its active form) can be accellerated by an appropriate substitution in the aromatic rings (e.g. of OH in 2-position). A hint to the practicability of this concept is the observation that the shift of the OH-group in the R,R/S,S configurated [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complex to the 3- or 2-position, respectively, leads to a marked elevation of the antitumor activity in the P 388 leukemia mouse test[10,11]. In an experiment with (R,R/S,S)-[1,2-bis(2-hydroxyphenyl)ethylenediamine] dichloroplatinum(II) (dosage: 9 x 13.2 µmol/kg, days 1 to 9) it was even possible to cure 4 out of 6 animals[11]. The substi- tution of OH-groups also confers an increase of water solubility of dichloro(1,2-diphenylethylenediamine) platinum(II) complexes, which is advantageous for their therapeutic use.

In the case of the R,S configurated diaquaplatinum(II) type (i.e. the therapeutically active form) the reaction with DNA could be improved by a diminution of the steric shielding of Pt. To this end one of the two phenyl rings in (R,S)-diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) salts must be replaced by a smaller residue.

A further possibility to optimize the antitumor activity of the (R,R/S,S)-[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes is the resolution into their enantiomers. Since DNA is a chiral substrate, platinum complexes, containing the enantiomers of the ligand, are endowed with different biological activities.

The three routes of molecule modification outlined above should be followed in further experiments to optimize the antitumor activity of [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes.

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Experimental Part

P 388 Leukemia[12]

The P 388 cell line was kindly provided by *Dr. Bogden* Laboratory of Experimental Oncology, *E.G.* and *G. Bogden* Laboratories, Worcester, Mass., USA.- Cells (10^6) were transplanted intraperitoneally into 7-9 weeks old female CD_2F_1-mice (Zentralinstitut für Versuchstiere, Hannover; Altromin^R and water ad libitum) with an average weight of 20 g. The test compounds were administered intraperitoneally at day 1, 5, and 9 after transplantation. The diaquaplatinum(II) complexes were dissolved in bidistilled water, the dichloroplatinum(II) complexes suspended in 50% polyethyleneglycol 400 (Fluca) as 0.9% NaCl solution. The median survival time of treated (6 mice/group) and control groups (10 mice/group) were determined, and the antitumor activity was expressed by % T/C.

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7 Corresponding to the stem cell concept, a subpopulation of tumor cells (i. e. the tumor stem cell) is responsible for tumor growth or regrowth after drug treatment and for the appearance of metastases[8]. Tumor stem cells have the capacity for producing a large number of progeny and for renewing themselves. Therefore, to obtain a curative cancer chemotherapy, the tumor stem cell population must be eradicated[9]. It is assumed that in the in vitro soft agar clonogenic assay (TCA) tumor stem cells are responsible for the formation of colonies. Thus, the TCA is supposed to have predictive value for possible in vivo drug activity.

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