[Chem. Pharm. Bull. 36(5)1895—1898(1988)]

Synthesis and Antitumor Activity of Riboflavin and Flavin Mononucleotide Pt(II) Complexes

KENJIRO SUZUKI,[a] MASAHIDE NOJI,[b] TAZUKO TASHIRO[c] and YOSHINORI KIDANI*,[b]

Department of Pharmacy, Tokai Teishin Hospital,[a] 2–17–5 Matsubara, Naka-ku, Nagoya 460, Japan, Faculty of Pharmaceutical Sciences, Nagoya City University,[b] 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan and Cancer Chemotherapy Center, Japanese Foundation for Cancer Research,[c] 1–37–1 Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan

(Received November 11, 1987)

Several Pt(II) complexes of riboflavin (RF) and flavin mononucleotide (FMN) with 1,2-cyclohexanediamine (dach) or 2-(aminomethyl)cyclohexylamine (amcha) isomers were prepared. The coordination sites of RF or FMN to Pt(II) ions were determined to be N(5) and O(6) with resultant chelate ring formation.

Antitumor activities of the dach or amcha Pt(II) complexes were tested against murine leukemia L1210 and all of them were antitumor-active. Pt(FMN)(*trans-l*-dach) exhibited the highest activity with a T/C% value of 326, with 4 cured mice out of 6. These results justify further testing.

Keywords——platinum(II) complex; antitumor activity; riboflavin; 1,2-cyclohexanediamine; 2-(aminomethyl)cyclohexylamine

We have prepared a variety of platinum(II) complexes containing 1,2-cyclohexanediamine (dach) and 2-(aminomethyl)cyclohexylamine (amcha) isomers as carrier ligands by exchanging leaving groups. The antitumor activities of these complexes were tested against murine leukemia L1210 or P388. The results were reported [1–5] and one of the Pt(II) complexes has been taken through a phase I clinical trial[6] and is now in a phase II clinical trial.

In this paper we will describe several newly prepared dach or amcha Pt(II) complexes containing riboflavin (RF) or flavin mononucleotide (FMN) as a leaving group. Their antitumor activities were tested against L1210. RF itself was reported to exhibit tumor-inhibiting action[7] and thus we were interested in synthesizing RF Pt(II) complexes in the expectation of synergism. In order to investigate the influence of amine ligands on the antitumor effects, ethylenediamine (en) and ammine Pt(II) complexes of RF or FNM were also synthesized.

Previously, Albert[8] had demonstrated metal complex formation between RF and metals by potentiometric titration and some other researchers confirmed his conclusions.[9,10] Albert pointed out the resemblance between RF and 8-hydroxyquinoline as regards chelate ring formation with metal ions and suggested that RF might coordinate through N(5) and O(4). Foye and Lange[11] reported separation of RF metal complexes from alkaline solution and the dark red Ag complex of RF was also prepared.[12,13] RF can coordinate to ruthenium, which belongs to the platinum group, through N(5) and O(4); its coordination sites were confirmed by X-ray crystallographic analyses of [Ru(10-methyl-isoalloxazine) $(NH_3)_4$]$(PF_6)_2$ H_2O.[14]

Experimental

Syntheses of Pt(II) Complexes

Pt (RF) (*trans-l*-dach)——PF (1.13 g, 3 mmol) was suspended in 400 ml of H_2O, and 1.30 g (3 mmol) of Pt $(NO_3)_2$ (*trans-l*-dach) dissolved in 100 ml of H_2O was added with heating. The mixture was heated on a water bath at 90 °C for 48 h with protection from light. After filtration, the filtrate was concentrated to 10 ml under reduced pressure and ethanol was added to the concentrate to give dark red precipitates. They were washed with acetone and dried *in vacuo* at 50—60 °C. *Anal.* Calcd for $C_{23}H_{31}N_7O_9Pt$: C, 36.95; H, 4.58; N, 13.11. Found: C, 37.14; H, 4.65; N, 12.77.

Pt (FMN) (*trans-l*-dach)——A solution of 1.30 g (3 mmol) of $Pt(NO_3)_2$(*trans-l*-dach) in 100 ml of H_2O was added to a solution of 1.54 g (3 mmol) of FMN (mono sodium) in 400 ml of H_2O. The mixture was stirred at room temperature with protection from light. After 48 h, it was concentrated to 25 ml under reduced pressure and ethanol was added to the concentrate to give dark red precipitates. They were washed thoroughly with ethanol and dried *in vacuo* at 50—60°C. *Anal.* Calcd for $C_{23}H_{31}N_6O_9$ Pt: C, 35.82; H, 4.32; N, 10.87. Found: C, 35.75; H, 4.44; N, 10.54.

RF and FMN Pt(II) complexes containing other diamines or ammonia were prepared by procedures similar to those described for Pt (RF or FMN) (*trans-l*-dach).

Paper chromatography was carried out on a paper filter (Toyo Roshi No. 50, 2.0 × 40 cm) with the upper layer of *n*-BuOH–CH_3COOH–H_2O (4 : 1 : 5) mixture as a developing solvent. RF and FMN were detected by fluorescence measurement and Pt(II) complexes by using 5% $SnCl_2$ solution.

Measurements——Infrared (IR) and electronic absorption (AB) spectra were measured with a Shimadzu IR-400 infrared spectrometer and a Hitachi 577 spectrophotometer, respectively.

Evaluation of Antitumor Activity——Antitumor activities of Pt(II) complexes were tested by means of the protocols for routine screening at the National Cancer Institute (Bethesda, Md.). L1210 cells (10^5) were transplanted intraperitoneally into CDF_1 mice on day 0, and the samples were given intraperitoneally on days 1, 5 and 9. From the mean survival times (day) of treated (T) and control (C) mice, T/C% values were calculated. Samples with T/C% values that exceeded 125 were evaluated as antitumor-active. A dose at which the T/C% value was less than 85 or weight loss of treated mice was greater than 4 g compared with that of control mice before day 5 was designated as a toxic dose.

Results and Discussion

Chemistry

Formation of diamine Pt(II) complexes with RF or FMN was confirmed by paper chromatography, and by the electronic absorption, fluorescence and IR spectra. In spite of using different diamines and ammonia, the RF or FMN Pt(II) complexes showed similar spectral behavior and mainly *trans-l*-dach Pt(II) complexes of RF or FMN will be discussed.

In order to identify complex formation, paper chromatography was employed to differentiate the Pt(II) complexes from starting materials. RF, FMN and $[Pt(NO_3)_2$ (*trans-l*-

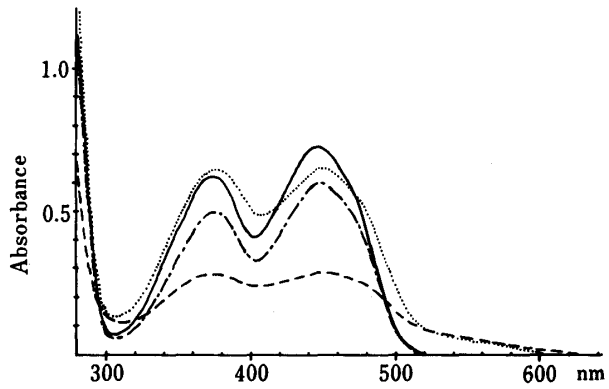

Fig. 1. Electronic Absorption Spectra of [Pt(RF)(*trans-l*-dach)]NO_3 and [Pt(FMN)(*trans-l*-dach)] together with Those of Free RF and FMN in H_2O

The concentration of each sample was 5×10^{-5} M. ------, Pt(RF)(*trans-l*-dach); – – – –, Pt(FMN)(*trans-l*-dach); ——, free RF; – · – · –, free FMN.

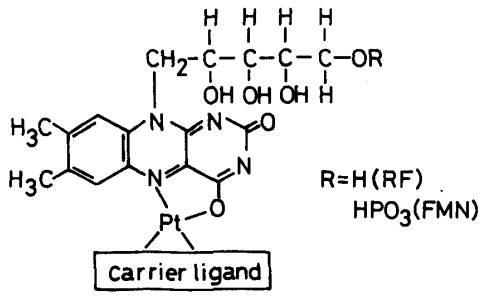

Fig. 2. Proposed Structure for Pt(II) Complexes of RF and FMN

TABLE I. Antitumor Activities of Pt(II) Complexes Containing Riboflavin or Flavin Monophosphate against Leukemia L1210

Complexes	Dose (mg/kg)							
	200	100	50	25	12.5	6.25	3.12	1.56
	T/C %							
[Pt(RF)(*trans-l*-dach)]NO_3				192 (1)	167	129	115	
[Pt(RF)(*trans-d*-dach)]NO_3		126	173	177	145	124		
[Pt(RF)(*cis*-dach)]NO_3	0	0	165	115	105	100		
[Pt(RF)(*cis-dl*-amcha)]NO_3	0	0	133 (1)	196	169 (1)			
[Pt(RF)(*trans-dl*-amcha)]NO_3			89	192	125			
cis-[Pt(RF)$(NH_3)_2$]NO_3		139	136	146	115	106		
[Pt(RF)(en)]NO_3	T110	122	116	109	101			
[Pt(FMN)(*trans-l*-dach)]		T170 (1)	326 (4)	253	185	124	125	115
[Pt(FMN)(*trans-d*-dach)]	0	131	161	121	109			
[Pt(FMN)(*cis*-dach)]	0	227 (2)	114	117	105			
[Pt(FMN)(*cis-dl*-amcha)]			134	112	119			
[Pt(FMN)(*trans-dl*-amcha)]			146	179	130			
cis-[Pt(FMN)$(NH_3)_2$]		106	107	96	102			
[Pt(FMN)(en)]	116	117	113	106	103			

Underlined figures indicate significant antitumor activity (T/C %≧125). T indicates toxicity. The numbers in parentheses indicate cured mice out of 6 mice in one group. L1210 cells (10^5 cells/mouse) were transplanted i.p. into CDF_1 mice, and the test samples were administered i.p. on days 1, 5 and 9.

dach)] used as starting materials showed spots at *Rf* values of 0.38, 0.20 and 0.61, respectively. The RF and FMN Pt(II) complexes of *trans-l*-dach showed spots at *Rf* values of 0.41 and 0.27, respectively, accompanied with disappearance of the fluorescence characteristic of RF or FMN. The quenching of the fluorescence was also confirmed by measuring the fluorescence spectra. Exciting RF or FMN with light of 440 nm wavelength gave a fluorescence spectrum with a maximum at 510 nm, which was completely eliminated in the spectrum of the corresponding Pt(II) complexes.

The color of mixed solutions of RF or FMN and Pt$(NO_3)_2$(*trans-l*-dach) changed gradually to dark red as the reaction proceeded and dark red precipitates were formed, clearly indicating Pt(II) complex formation. In fact, the AB spectrum of free RF exhibited absorption maxima at 374 and 446 nm, as shown in Fig. 1, while that of Pt(RF)(*trans-l*-dach) gave absorption maxima at 374 and 450 nm with a new broad band around 520—540 nm, which is responsible for the dark red color of the Pt(II) complexes. Pt(FMN)(*trans-l*-dach) also showed a broad band around 520—540 nm in its AB spectrum.

The IR spectrum of Pt(RF)(*trans-l*-dach) exhibited only one peak at 1650 cm^{-1} due to $\nu_{C=O}$, while that of free RF showed two peaks at 1720 and 1650 cm^{-1}, which were assigned to $\nu_{C(4)=O}$ and $\nu_{C(2)=O}$, respectively.[14] The disappearance of the peak at 1720 cm^{-1} indicates the enol form of the carbonyl group at C(4), suggesting O(4)–Pt(II) bonding. Since N(5) is located at a convenient position for chelate ring formation through N(5) and O(4), it is natural to conclude that RF coordinates Pt(II) ions through N(5) and O(4). The IR spectrum of Pt(FMN)(*trans-l*-dach) also showed similar behavior, and a proposed structure is shown in Fig. 2.

Antitumor Activity

Table I lists the antitumor activities of dach or amcha Pt(II) complexes containing RF or FMN against murine leukemia L1210 determined according to the NCl Pt Analog Study Protocol together with those of ammine and en Pt(II) complexes. Among RF Pt(II) complexes of dach isomers, *trans-l*-dach Pt(II) complex exhibited the highest activity with a

T/C% value of 192 at 25 mg/kg and one mouse was cured out of 6 mice. As regards the antitumor activities of RF Pt(II) complexes containing geometrical amcha isomers, *trans-dl*-amcha Pt(II) complex exhibited somewhat higher activity than *cis-dl*-amcha Pt(II) complex. Interestingly, Pt(RF)(en) did not show any activity against L1210 and *cis*-Pt(RF)$(NH_3)_2$ showed only a marginal effect. Among the FMN Pt(II) complexes examined, *trans-l*-dach Pt(II) complex exhibited the highest activity with a T/C% value of 326 at 50 mg/kg, curing 4 mice. However, ammine and en Pt(II) complexes were inactive.

The antitumor activities of Pt(II) complexes containing RF or FMN depended largely on the amine ligands used as carrier ligands. The results with Pt(FMN)(*trans-l*-dach) justify further testing.

Acknowledgments This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture (No.61125006). We are also grateful to Tanaka Kikinzoku Kogyo K. K. and the Fujisawa Foundation for financial supports. We thank Miss Makiko Hayashi for her assistance in part of the experimental work.

References

1) Y. Kidani, K. Inagaki and R. Saito, *J. Clin. Oncol. Hematol.*, **7**, 197 (1977).
2) Y. Kidani, K. Okamoto, M. Noji and T. Tashiro, *Gann*, **69**, 863 (1978).
3) Y. Kidani, M. Noji and T. Tashiro, *Gann*, **71**, 637 (1980).
4) M. Moji, K. Okamoto, Y. Kidani and T. Tashiro, *J. Med. Chem.*, **24**, 508 (1981).
5) K. Okamoto, M. Noji, T. Tashiro and Y. Kidani, *Chem. Pharm. Bull.*, **29**, 929 (1981).
6) G. Mathé, Y. Kidani, K. Triana, S. Brienza, P. Roband, E. Goldschmidt, E. Ecstein, R. Depax, M. Musset and J. L. Misset, *Biomedicine and Pharmacotherapy*, **40**, 372 (1986).
7) G. I. Kensler, K. Sugiura, N. F. Young, C. R. Halter and C. P. Rhodas, *Science*, **93**, 308 (1941).
8) A. Albert, *Biochem. J.*, **47**, 27 (1950).
9) T. R. Harkins and H. Freiser, *J. Phys. Chem.*, **63**, 309 (1959).
10) M. M. T. Khan and M. S. Mohan, *J. Inorg. Nucl. Chem.*, **35**, 1749 (1973).
11) W. O. Foye and W. E. Lange, *J. Am. Chem. Soc.*, **76**, 2199 (1954).
12) P. Bamberg and P. Hemmerich, *Helv. Chim. Acta*, **44**, 1001 (1961).
13) A. M. D. Macpherson and J. M. Ottaway, *Analyst* (London), **103**, 830 (1978).
14) M. J. Clarke and M. G. Dowling, *Inorg. Chem.*, **20**, 3506 (1981).
15) J. T. Spence and E. R. Peterson, *J. Inorg. Nucl. Chem.*, **24**, 601 (1962).