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Short communication

Synthesis and antitumor activity of a platinum (II)-doxorubicin complex

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Summary. A mixed platinum (II) complex with *tert*-butylamine and doxorubicin (cooordinated *via* the aminogroup) has been synthesized and tested for antitumor activity. The results indicate that the complex was active against doxorubibin-resistant P388 and cisplatin-resistant L1210 leukemias, while maintaining antitumor activity against sensitive parent lines.

Introduction

As a result of the success of doxorubicin (DX) and cisplatin in the clinical cancer chemotherapy, there has been an increase in interest in the development of new derivatives in both classes with more favorable properties, such as greater efficacy, reduced toxicity, broader spectrum of activity, and lack of cross-resistence to parent compounds. The latter property might result in therapeutic advantage in the treatment of human tumors that have became resistant to the antitumor effects of the parent drug or frequently develop drug resistance. Recently, the design of antitumor agents carrying multifunctional groups has been proposed as a possible approach to improve the effectiveness of known agents [9, 12].

Since cisplatin is characterized by lack of cross-resistance to anthracyclines, the aim of the study described in this paper was to examine the antitumor properties of a complex of platinum (II) with DX in sensitive and resistant tumor models. We also describe the synthesis of the complex, which was prepared in our laboratories.

Materials and Methods

Drugs: DX was obtained from Farmitalia-Carlo Erba (Milan, Italy); cisplatin was obtained according to Kauffman and Cowan [8]. Potassium *tert*-butylaminetrichloro platinate ($K[PtCl_3(tba)]$) was prepared as reported [1]; yields were in the range 50%–60%. The mixed platinum (II)-DX and *tert*-butylamine (tba) complex was obtained by mixing water solutions of equimolar amounts of DX. HCl and $K[PtCl_3(tba)]$. The mixture was kept at room temperature under a nitrogen atmosphere and in the dark for 10 h and evaporated to dryness under reduced pressure. The residue was treated with chloroform/methanol (8/2, v/v) and KCl was filtered off. The compound was precipitated by the addition of a large amount of diethyl ether to the solution. Satisfactory elemental analyses (C, H, N, Pt) were obtained.

Tumor models and antitumor testing. DBA/2 and BDF1 mice (weighing between 18 and 22 g) were obtained from Charles River Laboratories (Calco, Italy).

L1210 and P388 leukemias were maintained in ascitic form by serial i.p. passage of 10^5 and 10^6 cells/mouse, respectively, in DBA/2 mice. The experiments with L1210 and P388 leukemias were carried out in BDF1 female mice inoculated with the same number of cells. The P388 leukemia subline resistant to DX (P388/DX) was developed and established by Dr. F. M. Schabel (Southern Research Institute, Birmingham, Ala.) and was maintained in BDF1 mice by weekly i.p. inoculation of 10^7 cells per mouse. The mice used for maintenance of P388/DX were treated i.p. with 6 mg/kg of DX 2 days after transplantation. Experiments with P388/DX were carried out in BDF1 mice inoculated with 10^6 cells per mouse. The L1210 leukemia subline resistant to cisplatin (L1210/CDDP) was obtained from Frederick Cancer Research, (NCI, Md.) and was maintained by weekly i.p. passages (10^5 cells/mouse) in DBA/2 mice routinely receiving i.p. 4 mg/kg of cisplatin on day 4 after tumor inoculation. Experiments with L1210/CDDP were carried out in BDF1 mice inoculated with the same number of cells.

In all tumor models, the drugs were administered i.p. as a single dose (in a volume of 0.1 ml/10 g) on day 1 after tumor cell transplantation. Animals were observed daily and experiments were terminated on day 60. Mice alive on day 60 were described as "long-term survivors" (LTS). Comparative antitumor effects were determined from % T/C, defined as the median survival time (MST) of dying mice only in the treated group (T), divided by the MST of the untreated control group.

Results and Discussion

Complexes derived by the interaction between DX and cisplatin [14] or chloroplatinate (II) [11] have already been reported. The compound described in this paper, however, is designed in such a way that a pharmacologically active moiety *cis*-$PtCl_2$ is linked to an inert amine (A) and a mole-

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Table 1. Comparison of the effects of doxorubicin and platinum-doxorubicin complex on P388 and L1210 leukemias

Tumors	Drug[a]	Dose (μmol/kg)	T/C (%)	LTS[b]	Toxic deaths
P388[c]	CDDP	23	246	0/10	1/10
	K [$PtCl_3$(tba)]	40	211	0/10	0/10
	DX	17.3	305 (266, 344)	7/19	0/19
		26	300	7/10	0/10
	Pt-DX	17.3	363 (360, 366)	8/19	0/19
		26	388	5/10	0/10
L1210[d]	CDDP	23	175	1/8	0/8
	K [$PtCl_3$(tba)]	48.3	137	0/8	0/8
	DX	17.3	187	0/9	0/9
		24	200	1/9	1/9
	Pt-DX	17.3	187	0/9	0/9
		24	187	0/9	0/9
		33.8	225	0/9	1/9

[a] CDDP, cisplatin; K [$PtCl_3$(tba)], potassium *tert*-butylaminetri-chloroplatinate (II) (i.e., the synthetic precursor of Pt-DX); DX, doxorubicin; Pt-DX, platinum-doxorubicin complex
[b] LTS, long-term survivors
[c] Median survival time of control mice was 9 days in two experiments
[d] Median survival time of control mice was 8 days

cule of DX *via* its amino group. The synthetic strategy is as follows:

$K[PtCl_3(tba)] + DX \cdot HCl \rightarrow$ *cis*-$[PtCl_3(tba)\ (DX)] + KCl + HCl$.

Since Cl has a stronger *trans* labilizing influence than an amine [3] the *cis* configuration of the product is ensured, as confirmed by the appearance of two infrared bands (320 and 330 cm^{-1}, polyethylene disk) attributable to the Pt-Cl stretching modes [13]. The remaining bands in the infrared spectrum are identical to those of DX · HCl; in particular, the bands of the hydroxyanthraquinone moiety are unaffected, showing that these groups do not participate in the Pt binding. This is also confirmed by the fact that both electronic and circular dichroism spectra of the complexes are superimposable on that of DX · HCl. Binding of a metal to the hydroxyquinone function should, in fact, change all these spectra [5]. The remaining possibility is that Pt is bound to DX *via* the deprotonated 3'-amino group of danosamine. Hydrochloric acid is in fact liberated during the reaction. Moreover, the chemical shift in the ^{195}Pt n.m.r. spectrum (−1920 ppm relative to H_2PtCl_6, D_2O solution) is in the range expected for Pt (II) bound to two nitrogen and two chlorine atoms [6]. By the same procedure, a compound with other amines can be obtained, but yields were so poor that in vivo tests were omitted.

Table 2. Comparison of the effects of doxorubicin and platinum-doxorubicin complex on P388/DX and L1210/CDDP

Tumor	Drug[a]	Dose (μmol/kg)	T/C (%)	Toxic deaths
P388/DX[b]	CDDP	16.7–23	183 (175–191)	4/18
	DX	17.3	100	0/9
		26	100	0/9
	Pt-DX	17.3	230	0/9
		26	170	2/9
L1210/CDDP[c]	CDDP	23	100	0/10
		33	100	1/10
	DX	17.3	145[d]	0/10
		26	159[d]	1/10
	Pt-DX	17.3	136	0/9
		24	145	0/10

[a] CDDP, cisplatin; DX, doxorubicin; Pt-DX, platinum-doxorubicin complex
[b] Median survival time of control mice was 10 days
[c] Median survival time of control mice was 11 days
[d] Two long-term survivors were observed

The DX-Pt complex was evaluated against two i.p. murine leukemias (P388 and L1210), sensitive to either DX and cisplatin, and two sublines, one with acquired resistance to DX (P388/DX) and the other to cisplatin (L1210/CDDP). The activity was compared with those of free DX, at equimolar doses. Cisplatin was also included for comparison in each experiment at its optimal dose, as a reference compound.

When examined against sensitive leukemias (Table 1), the activity of Pt-DX complex was comparable to that of free DX, since the drugs produced similar increase in the survival time of the animals and a similar number of LTS. In both experimental models, the increase in survival time obtained with optimal doses of cisplatin was somewhat lower than that observed with anthracycline.

Since in DX-related anthracyclines the presence of a free aminogroup is recognized as an important structural requirement for optimal activity [2, 15], the complete retention of drug activity in a complex in which Pt is linked to the amino function suggests that the mechanism of action of this derivative is more complex than that of free anthracyclines, as also proposed for a new group of N-modified derivatives [7].

In the treatement of resistant sublines (Table 2), the Pt-DX complex showed absence of cross-resistance. As expected, no cross-resistance was observed between DX and cisplatin. Against P388/DX and L1210/CDDP, the activity of the complex was similar to that of cisplatin and DX, respectively.

The results presented in this paper show that we have been successful in preparing an analogue active in resis-

tant tumor syytems, based on the rationale that anthracyclines and platinum compounds are not cross-resistant. This derivative might be of clinical relevance because of the interest in combining DX and cisplatin in the treatment of human solid tumors [4, 10]. However, we have not yet carried out a detailed comparative study on the combination of these drugs and therefore cannot comment on the possible therapeutic advantage combination might have. However, these preliminary results indicated that the binding of platinum to DX did not result in an increase of drug toxicity, since the anthracycline doses tolerated were similar in the free or Pt-bound form.

Further studies are needed to document the preclinical efficacy and pharmacological value of the Pt-DX complex in different experimental tumor systems using different routes of administration.

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References

1. Bersanetti E, Pasini A, Pezzoni G, Pratesi G, Savi G, Supino R, Zunino F (1984) Antitumor complexes of platinum with carrier molecules. 2. Mixed complexes of amino acids and *tert*-butylamine. Inorg Chim Acta 93: 167
2. Brown JR (1983) New natural, semisynthetic anthracycline drugs. In: Molecular aspects of anti-cancer drug action, S. Neidle and M. J. Waring eds, MacMillan p. 57
3. Cotton FA, Wilkinson G (1972) In: Advanced Inorganic Chemistry. 3rd ed. J. Wiley, London, p 667
4. De Gramont A, Drolet Y, Lavoie A, Painchaud M, Blouin R, Tessier C, Quellet P (1985) Adriamycin and *cis*-platinum in advanced ovarian cancer. Eur J Clin Oncol 21: 665
5. Greenaway FT, Dabrowiak JC (1982) The binding of copper ions to daunomycin and adriamycin. J Inorg Biochem 16: 91
6. Ismail MI, Sadler PJ (1983) ^{195}Pt- and ^{15}N-NMR studies of antitumor complexes. ACS Symp Series 209: 171
7. Johnston JB, Glazer RI (1983) Cellular pharmacology of 3'-(4-morpholinyl) and 3'-(4-methoxy-1-piperidinyl) derivatives of 3'-deaminodaunorubicin in human colon carcinoma cells in vitro. Cancer Res 43: 1606
8. Kauffman GB, Conan DO (1963) *Cis*- and trans-dichlorodiammineplatinum (II. Inorg Synth 7: 239
9. Khaled MA, Morin RD, Benington F, Daugherty JP (1984) 2,4-Diamino-6-(bis-2-chloroethyl)aminomethyl pteridine. Cancer Chermother Pharmacol 13: 73
10. Klippstein TH, Mitrou PS, Kochendorfer KJ, Bergman L (1984) High-dose adriamycin (ADM) and *cis*-platinum (DDP) in advanced soft-tissue sarcoma and invasive thymomas.. A pilot study. Cancer Chemother Pharmacol 13: 78
11. Manake RC, Wolf W (1981) Cisplatinum compounds and analogues. In: Radiopharmaceuticals: Structure-acitivity relationship. Proc Symp, Grune & Stratton Inc., New York, p 183
12. Meischen SJ, Gale GR, Naff MB (1982) Synthesis and antitumor acitivity of N-phosphonacetyl-L-aspartato (1,2-diamminocylohexane)platinum (II). J Clin Hemat Oncol 12: 67
13. Nakamoto K (1970) In: Infrared spectra of inorganic and coordination compounds. 2nd ed. Wiley-Interscience, New York, p 157
14. Yolles S, Roat RM, Sartori MF, Washburne CL (1982) Controlled release of anticancer agents that are complexes of *cis*-diamminedichloroplatinum(II) and alpha-hydroxyquinones. In: ACS Symposium Series 186: 223
15. Zunino F, Di Marco A, Zaccara A (1979) Molecular structural effects involved in the interaction of anthracyclines with DNA. Chem-Biol Interactions 24: 217

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