Antitumor Complexes of Platinum with Carrier Molecules.
3.* Cytotoxicity of some Platinum Amino Acid Complexes against Cisplatin-sensitive and Resistant L1210 Leukemia Cells

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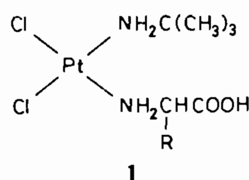
We have recently reported the synthesis and the biological activity of the cisplatin† analogues **1** in which the reactive moiety *cis*-$PtCl_2$ is coordinate to tert-butylamine and to the amino group of a variety of amino acids [1], on the rationale that tba increases the liposolubility of the compounds, while



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amino acids can act as carriers because of the high requirement for nutrients by tumor cells [2, 3]. The binding of carrier molecules to antitumor agents is expected to decrease their toxicity because of a selective transport to tumor tissues. Amino acid derivatives of alkylating agents [4] and platinum [5, 6] have indeed been reported, although with conflicting results.

Antitumor tests have shown that **1**-glyH, **1**-D-alaH, **1**-L-thrH, and **1**-L-serH display some activity against murine P388 and L1210 leukemias [1]. Although both activity and potency were found to be lower than that of cisplatin, we are continuing to investigate these compounds because we believe that an understanding of the mode of action of a drug (or the reasons for its low activity) can give some hints for the design of new superior analogues. For instance, recent studies have shown that the low activity of compounds **1** could arise, *inter alia,* from a steric hindrance in the reaction of **1** with DNA [7]. We report here some new observations on the role of the amino acid.

The cytotoxic activities of **1** against sensitive and cisplatin-resistant L1210 leukemia cells are reported in Table I, and compared with the activity of cis-

*Part 2 is ref. 1.

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†Abbreviations. cisplatin, *cis*-dichlorodiamminoplatinum(II); tba, tert-butylamine; aaH, N-coordinate amino acid; the derivatives of 1 are abbreviated 1-glyH; 1-L-alaH etc.; L1210/DDP, cisplatin resistant L1210 leukemia cells.

TABLE I. Cytotoxicity of *cis*-[$PtCl_2$(aaH)(tba)] Against L1210 and L1210/DDP Leukemia Cells[a]

Compound	ID_{50} (μmol/ml)		C (μmol/ml)[b]	Resistance index[c]
	L1210	L1210/DDP		
1-L-serH	12.6	24.3	0.29	1.9
1-D-serH	12.0	38.0	abs.	3.2
1-D,L-serH	15.5	28.1	abs.	1.8
1-L-pheH	16.6	20.8	0.10	1.3
1-D-pheH	26.8	29.7	abs.	1.1
1-L-leuH	25.5	18.0	0.38	0.7
1 L alaH	12.6	27.3	abs.	2.2
1-L-alaH	16.8	37.3	0.15[d]	2.2
1-L-methH	48.1	50.2	0.10	1.0
1-L-aspnH	8.3	22.2	0.43	2.7
Cisplatin	0.3	7.0		23.0
K[$PtCl_3$(tba)]	3.5	15.3		4.4

[a]L1210 and L1210/DDP cells were obtained according to ref. 8. 100 × 10^3 cells/ml were incubated with a range of drug concentrations for three days at 37 °C, under a 5% CO_2 atmosphere, in RPM 1640 (Synthetic Liquid Media) with 10% fetal calf serum and counted with a Coulter Counter. ID_{50} values were obtained by dose/response curves. [b]C, concentration of the corresponding amino acid in the culture medium. [c]Ratio between the ID_{50} values for the resistant and sensitive lines. [d]Concentration of L-alanine (μmol/ml) added.

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platin and of K[$PtCl_3$(tba)] (the synthetic precursor of **1**) as reference compounds. We wish to stress the following points:

(i) As expected there is no precise correlation between *in vivo* antitumor activity and cytotoxicity, even on the same line. For instance the ID_{50} values of **1**-L-serH and **1**-L-pheH are comparable (Table I), but only the former has been found to be active *in vivo* [1].

(ii) In agreement with the already noted relevant activity of trichloroamineplatinum compounds (neutral [9] and anionic [1, 10]), the cytotoxicity of K[$PtCl_3$(tba)] is rather high; indeed it is higher than those of **1**. It seems that substitution of a chloride for an N-coordinate amino acid lowers cytotoxicity.

(iii) The presence of an N-coordinate amino acid, however, produces compounds characterized by comparable activity on the two lines. K[$PtCl_3$(tba)] and, of course, cisplatin, are less cytotoxic against L1210/DDP than against L1210 leukemia cells, whereas the ID_{50} of compounds **1** are only slightly higher towards the resistant line (compare resistance indexes in Table I).

(iv) L-alanine is absent in the culture medium (RPM 1640, Synthetic Liquid Media), but addition of this free amino acid does not affect appreciably the ID_{50} value of **1**-alaH. This observation, together with (ii), casts some doubts on the role of these N-coordinate amino acids as carriers.

(v) L1210 leukemia cells have been reported to be auxotrophic for L-methionine [11], but this fact has recently been questioned [12]. In any case **1**-L-methH has shown the lowest cytotoxicity.

(vi) In contrast with observation (iv), the relatively high cytotoxicity of **1**-L-aspnH is in accordance with the reported high requirement of leukemia cells for L-asparagine [13]. Note, however, that **1**-L-aspnH shows the highest cross resistance in the series of **1**.

For **1**-L-aspnH we have also found that there is neither synergism nor competition with L-asparaginase. Addition of ID_{25} and ID_{50} of this enzyme* to the ID_{50} of **1**-L-aspnH produced only additive effects. The same was found also for cisplatin.

All these results seem to suggest that in this type of compounds, N-coordinate amino acids contribute neither to cytotoxicity, nor, presumably, to preferential uptake by cells, with the possible exception of **1**-L-aspnH. Such a lack of preferential uptake is in contrast with what was reported for the phenylalanine mustard melphalan, which is probably transported into the L1210 cells by an amino acid transport system [14]. It must be pointed out, however, that in melphalan the amino group is free and not coordinated as in **1**. A different explanation could be that compounds **1** inhibit the amino acid transport systems in L1210 cells, as reported for cisplatin [15]. These compounds could therefore be self-inhibitors.

*Under our experimental conditions (see Table I) ID_{25} and ID_{50} for L-asparaginase were found to be 0.01 u and 0.1 units, respectively, against either line.

TABLE II. Pt uptake by L1210 and L1210/DDP Cells Treated with **1**-L-serH and Cisplatin[a,b]

Dose (μmol/ml)	L1210		L1210/DDP	
	Pt, found (μmol)	Found/Added (%)	Pt, Found (μmol)	Found/Added (%)
Cisplatin				
160			5.33	0.11
80	5.50	0.20	2.33	0.097
40	1.74	0.15	0.98	0.082
20	0.9	0.15	0.51	0.085
10	0.74	0.24	0.28	0.093
5	0.22	0.15		
1-L-serH				
183	10.7	0.19	8.51	0.15
122	7.7	0.20	6.46	0.18
81	4.10	0.17	3.85	0.16

[a]Cells (60×10^6 in 30 ml) were incubated for 2 h with the drugs, centrifuged, washed three times with physiologic saline solution, and Pt content was analyzed by flameless atomic absorption spectroscopy. Other details are as in footnote [a], of Table I. [b]Under these conditions (2 h treatment) ID_{50} values were found to be approximately ten times higher than those reported in Table I.

We have therefore undertaken a study on the uptake of **1** and cisplatin by L1210 and L1210/DDP cells in culture. Some very preliminary results show that the uptake of **1**-L-serH by L1210 cells is not higher than that of cisplatin (Table II). For cisplatin our results are in agreement with those of a recent report [16]. The percent of Pt taken up by the resistant line is lower than that accumulated by the sensitive line, and this difference is much more pronounced for cisplatin.

The mechanism of resistance to cisplatin is at present unknown, although a rise of the intracellular concentration of protein and non-protein thiol groups has been suggested to contribute to such a resistance [17, 18]. However, it has been proposed that cisplatin uptake by the cells depends on some membrane carrier system [19]. A lower intracellular Pt accumulation in the resistant line, compared to the sensitive one, suggests that an alteration of this drug transport could be involved in resistance. A mechanism involving membrane alteration, which results in a reduced drug accumulation, has also been proposed to contribute to resistance to melphalan [19] and anthracyclines [20]. For cisplatin this hypothesis seems to be supported by recent results [21].

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