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STRUCTURE-ACTIVITY CONSIDERATION OF NOVEL ANTICANCER PLATINUM PYRIMIDINE
"GREENS"

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Platinum pyrimidine greens are very effective against L1210 cells, but the blues are inactive. A clear relationship between the activity and size of Pt-green molecules has been observed; smaller molecules with up to Pt-decanuclear complexes are much more active than larger ones. Formation of macrocells by the greens have been found for the first time with L1210 cells. The more active the green complex is, the denser is the population of the macrocells. These findings could be related to the membrane permeability.

KEYWORDS — platinum uridine green; L1210 cell anticancer activity; macrocell formation; activity-molecular size relation; inactive platinum uridine blue

During the course of our extensive studies on antitumor platinum pyrimidine oligomer complexes,¹⁻⁵⁾ we have discovered that the effect of platinum "green" species isolated by gel filtration as a minor product on the life span of mice bearing L1210 leukemia was exceedingly high.¹⁾ In the earlier investigations,⁶⁾ the materials used for biological assays were not always sufficiently purified. Therefore, it is very probable that the activity of platinum blues reported so far has been caused by green species included in the samples. To test the assumption, efficient and selective synthesis of platinum greens was essential. This has been achieved very recently by the authors.^{2,4)} Since then we have been examining the structure-activity relationships using L1210 cells in tissue culture, and describe here the new results.

The platinum uridine greens investigated in this work have been prepared from aquated cis-diiododiammineplatinum(II)⁷⁾ and uridine treated with hydrogen peroxide as mentioned in a preceding paper.⁴⁾ Their molecular range from 8 to 28 as numbers of Pt in the molecule (platinum octamer to octacosamer). These are summarized in Table I, and the microanalytical data are given in the reference part.⁸⁾

Biological activities were determined by examining the inhibition of growth of L1210 cells in tissue culture. The cells were cultured in the presence or absence of test samples (final concentration of 5 or 10 μ g/ml) at 37 °C in

culture medium (GIBCO RPMI 1640) containing kanamycin and 10% of fetal bovine serum. The ratios of growth of the treated vs. control cells (initially employed 1×10^5 cells/ml, and grew to 1.4×10^6 cells/ml after 4 days) were determined. The bioassay results are also summarized in Table I.

Table I. Growth Inhibition of L1210 Cells in Tissue Culture by Platinum Uridine Greens

Sample ^{a)}	Synthesis ^{b)}	Molecular size Pt:Ud:NH ₃ ^{c)}	Growth inhibition, %		Ratio of macrocells ^{e)}	Remarks ^{f)}
			5 μ g/ml	10 μ g/ml ^{d)}		
1	A	10 : 6 : 20	78.0	92.8	-	-
2	"	11 : 6 : 22	59.2	93.7	-	-
3	"	11 : 9 : 20	-	77.1	0.43	84.1
4	B	10 : 8 : 19	-	84.8	1.34	91.3
5	"	9 : 4 : 16	-	99.3	3.0	99.8
6	A	11 : 8 : 21	-	91.6	2.05	97.3
7	B	8 : 5 : 15	-	99.2	3.25	99.8
8	A	13 : 13 : 20	32.4	68.3	-	-
9	B	14 : 14 : 23	12.3	51.9	-	-
10	"	13 : 12 : 23	20.9	69.2	-	-
11	"	16 : 16 : 28	-	35.5	-	-
12g)	B	28 : 28 : 29	-	-1.9	0.0035	-1.5

a) Prepared in the same manner described in ref. 4. b) A: further details and microanalytical data are given in reference 8. B: synthesized in the previous work; see ref. 4. c) Calculated from elemental analysis results. d) Sample concentration used for the activity study. e) Ratios of macro- and normal-L1210 cells with a 10 μ g/ml dose after 4 days at 37°C. See text. f) Growth inhibition at a 10 μ g/ml dose determined excluding macrocells formed. See text. g) Pt-blue complex; prepared by air oxidation.

At a 10 μ g/ml dose, platinum uridine greens were considerably active, but the corresponding blue material again clearly had no effect on the cell growth (actually, platinum blue sample had some promoting effect on the cell growth; 12 in Table I). These observations are thoroughly consistent with our earlier discoveries with *in vivo* experiments using CDF₁ mice.¹⁾

It turned out that, among the green materials examined here, different preparations showed different activities; growth inhibition was distributed from 35.5 to 99.3% as shown in the Table. If we take molecular size into consideration, there seems to be a reasonable relation to the activity. Figure 1 shows the percentage of growth inhibition relative to molecule size (shown as numbers of platinum included). Clearly, smaller molecules are more active than larger ones. And molecules which contain up to 10 platins (up to Pt-decanuclear complexes; see samples 1, 4-7) are the most effective against L1210 cells. To determine the best complex, related studies with lower molecules are currently in progress, which will be reported in detail later in a full paper. A wholly parallel tendency occurs with a smaller dose (5 μ g/ml), though lesser efficiency resulted (Table I & Fig. 1).

Fig. 1. A Relationship between Molecular Size and Activity against L1210 Cells in Tissue Culture
(○) with a 10 µg/ml dose. (●) with a 5 µg/ml dose.

There were considerable numbers of macrocells which were approximately twofold longer in diameter than the normal L1210 cells. The numbers of both cells were counted separately, and the ratios of macro- vs. normal-cells were calculated. The data are listed in Table I. Clearly the more active the material is, the denser is the population of the macrocells. For example, although almost no macrocells were detected in the presence of blue complex (sample 12), over three quarters of the surviving cells (even though only 0.7-0.8% of the cells survived compared with the control) were macrocells in the most efficient examples (see samples 5 and 7). In the last column of the Table I, growth inhibition determined excluding such macrocells are listed for reference.

Our present findings may be the first direct evidence of macrocell formation in animal cells with platinum greens. But two decades ago, formation of macrocells (up to 300 times larger than the normal bacteria) was reported in growth experiments with *E. coli* under electric fields using Pt-electrodes.¹⁰⁾

Thus we have concluded that without doubt platinum uridine greens are active against L1210 cells, but that the corresponding blues are inactive. The activity differences recognized among the green complexes examined here are explainable by an evident relationship to the size of the molecules. Formation of macrocells has been perceived in the presence of Pt-greens for the first time with L1210 cells, which seems to be concerned with the mode of biological reactions. The above results may be related to the permeability of the membranes. This is one of the most important targets to be studied.

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- 8) Microanalysis results of platinum uridine greens synthesized in this work;
For sample 1: Preparation condition and yield; [2°C, 3% H₂O₂, 0.1 M, 3 wk, 44.9%]; Anal. Calcd for [Pt₁₀(C₉H₁₁N₂O₆)₆(NH₃)₂₀(OH)₂(H₂O)₆](SO₄)₈/8H₂O: C,13.50; H,3.27; N,9.33; S,5.34; Pt,40.60; Found: C,13.42; H,3.18; N,9.15; S,5.25; Pt,40.48.
For 2: [2°C, 6% H₂O₂, 0.1 M, 3 wk, 17.4%]; Calcd for [Pt₁₁(C₉H₁₁N₂O₆)₆(NH₃)₂₂(OH)₃(H₂O)₇](SO₄)₉/12H₂O: C,12.38; H,3.33; N,9.09; S,5.51; Pt,40.97; Found: C,12.36; H,3.17; N,8.99; S,5.44; Pt,40.70.
For 3: [2°C, 1% H₂O₂, 0.1 M, 4 wk, 29.5%]; Calcd for [Pt₁₁(C₉H₁₁N₂O₆)₉(NH₃)₂₀(OH)₃(H₂O)₃](SO₄)₇/8H₂O: C,17.38; H,3.31; N,9.51; S,4.01; Pt,38.34; Found: C,17.21; H,3.19; N,9.50; S,3.94; Pt,38.47.
For 6: [40°C, 1% H₂O₂, 0.05 M, 48 hr, 50.1%]; Calcd for [Pt₁₁(C₉H₁₁N₂O₆)₈(NH₃)₂₁(OH)₂(H₂O)₅](SO₄)₈/6H₂O: C,15.87; H,3.24; N,9.51; S,4.71; Pt,39.38; Found: C,16.14; H,3.13; N,9.34; S,4.51; Pt,39.02.
For 8: [75°C, 1% H₂O₂, 0.1 M, 45 min, 19%]; Calcd for [Pt₁₃(C₉H₁₁N₂O₆)₁₃(NH₃)₂₀(OH)₃(H₂O)₃](SO₄)₇/13H₂O: C,19.93; H,3.40; N,9.14; S,3.18; Pt,35.97; Found: C,19.63; H,3.25; N,9.15; S,3.18; Pt,36.27.
- 9) Calculated from unburned ash.
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