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In Vitro Antitumor Activity of Platinum Pyrimidine Greens Obtained by One-pot Synthesis on L1210 Cells

HIROAKI OKUNO*, TAKEHIKO SHIMURA, TOSHIMASA UEMURA, HIROSHI NAKANISHI and TAKENORI TOMOHIRO

National Chemical Laboratory (NCLI), Tsukuba, Ibaraki 305, Japan

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Platinum pyrimidine 'greens' are an important new family of antitumor drugs with distinctly different properties from the well-known platinum blues. For example, as we reported quite recently, the green compound is a potent inhibitor of the growth of mouse leukemic L1210 cells both *in vivo* [1] and *in vitro* [2, 3], whereas the corresponding blue materials are not. The low yield of the original synthesis has severely limited further investigation of these potentially promising therapeutic agents. Consequently considerable effort has been devoted to the development of better synthetic routes to platinum pyrimidine 'greens' [3–5].

As a result, we have developed a convenient one-pot reaction based upon *in situ* generation of hydrolysis products from *cis*-diiodiammineplatinum(II) during the reaction [6]. We report herein results on the biological activity of the resulting platinum green compounds against L1210 cells *in vitro*. We also discuss the influence of the ligands and of the preparation conditions on the biological activity of the Pt greens.

Experimental

Synthesis of the Platinum Greens

A series of *cis*-diammineplatinum green compounds with uridine, uracil, 5-fluorouracil, uridine 5′-monophosphate (UMP) and thymidine were prepared according to the previously reported procedures via a one-pot reaction. Optical spectroscopy and elemental analysis have established the identity of the product. While no X-ray data have yet been obtained, the products show the characteristic green color of the platinum uridine greens (λ_{max} = 265 and 722 nm in 10 mM H_2SO_4). Elemental analysis gives information on how reaction conditions influence the degree of oligomerization [6] (Table 1).

*Author to whom correspondence should be addressed (formerly Yohmei Okuno).

^{13}C NMR spectra (JEOL GX 400 spectrometer) indicate participation of the C-2 and C-4 carbonyl groups in the complex bonding between the Pt atom and the uridine base. Both C-2 and C-4 resonances shift substantially from the corresponding signals in the original uridine (about 7 ppm for C-2 and 10 ppm for C-4). The ^{13}C NMR spectrum of Pt green (run 6) gave three peaks for each C-2 and C-4 carbonyl carbons, two peaks for each C-1′, C-2′, C-3′, C-5′ and C-5 carbons, and only one peak for C-4′ and C-6 carbons in the uridine group. Three isomeric structures are expected: two H–H (head to head configuration of uridines) and one H–T (head to tail configuration). The ratio of H–T to H–H is approximately 0.7.

Biological Activity

The growth inhibitory activity of each platinum green was tested with mouse leukemic L1210 cells *in vitro*. Each tumor cell line (1×10^5/ml; Gibco RPMI 1640 medium containing kanamycin and 10% fetal bovine serum) was incubated in the presence or absence of compounds for 72 h at 37.6 °C. Then the numbers and size distributions of the cells were determined by a Coulter Counter ZM. The cells between 6.4 and 16 μm in diameter were counted. Percent inhibition was determined as follows: % inhibition = [1 – (cell numbers of sample well)/(cell numbers of control well)] × 100.

Results and Discussion

The IC_{50} (concentration at 50% inhibition of cell growth) values for these compounds are summarized in Table 1 as well as the *MIC* (minimum inhibitory concentration) values.

All platinum greens synthesized via the one-pot reaction exhibit remarkable growth inhibitory activities against L1210 cells. Although both the larger and smaller Pt greens have similar *MIC* values (1–2 μM), the smaller ones show higher inhibitory activity (IC_{50} values), as shown in Fig. 1.

Comparison of samples prepared at 40 and 75 °C clearly shows the greater activity associated with the smaller molecules. The 40 °C sample was much more active at low concentrations. HPLC reveals that this more active sample contains a greater proportion of low molecular weight Pt uridine greens. While both samples show peaks with retention times of 15.1 and 17.2 min (TSK gel G2500PW$_{XL}$, eluted with 10 mM H_2SO_4, pH 1.8, elution rate 0.5 ml/min), these occur in ratios of 1:14 and 5:1, respectively. Thus the greater activity of the 40 °C sample presumably derives from a greater proportion of the low molecular weight product.

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TABLE 1. Inhibitory Effects of Platinum Pyrimidine Greens Obtained by One-pot Reaction on the Growth of L1210 Cell Line *In Vitro*[a]

Run	Sub[b]	Pt:Sub:NH_3[c]	Molecular weight[c]	IC_{50}[d] (nM)	*MIC*[e] (μM)	Sub/Pt (%)
1	Uridine	8:5:14	3575	159	1.9	63
2	Uridine	9:6:17	4302	160	1.5	67
3	Uridine	8:6:14	3943	114	1.6	75
4	Uridine	8:3:16	3316	22	1.7	38
5	Uridine	12:9:21	5718	189	1.3	75
6	Uridine	12:8:22	5704	191	1.3	67
7	Uracil	12:3:21	4366	3.9	1.3	25
8	5-FU[f]	15:7:29	5567	181	1.5	47
9	UMP[g]	5:3:9	2376	1052	3.6	60
10	Thymidine	11:4:19	4504	138	1.7	36
11	Uridine	24:26:31	13003	369	0.85	108

[a]Each tumor cell line (1×10^5/ml; Gibco RPMI 1640 medium containing kanamycin and 10% FBS) was incubated in the presence or absence of compounds for 72 h at 37.6 °C. [b]Substrate. [c]Calculated from elemental analysis. [d]IC_{50} was given as the concentration at 50% inhibition of cell growth. [e]Minimum inhibitory concentration. [f]5-Fluorouracil. [g]Uridine 5'-monophosphate.

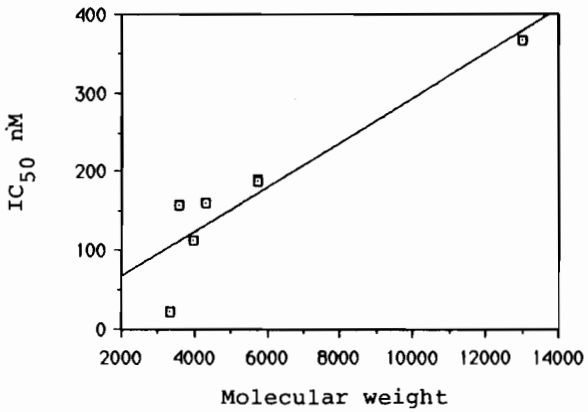

Fig. 1. A relationship between IC_{50} and molecular weight in platinum uridine greens. Regression coefficient = 0.92.

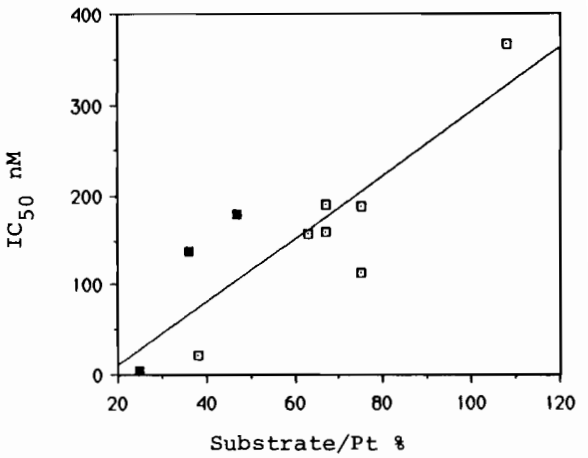

Fig. 2. Plots of IC_{50} and ratio of pyrimidine substrate to platinum in the molecule: (⊡) platinum uridine greens; (■) platinum greens with other pyrimidines. Regression coefficient = 0.85 (0.98 for platinum uridine greens only).

In addition, the 40 °C sample is also noteworthy for its low Ud/Pt ratio (38 and 67%, respectively for runs 4 and 6), which may result in more reactive sites on Pt at which DNA can bind. The same explanation may also apply to the remarkable activity of platinum uracil green which has U/Pt = 25%. Figure 2 shows a relationship between the IC_{50} value (in nM) and the ratio of the substrate to platinum. In the Pt uridine green series, a clear relation is obvious. Platinum greens with uracil, 5-fluorouracil and thymidine exhibit similar behavior. The large deviation from this relation observed in UMP may reflect inhomogeneity in the structure and in the nature of this compound. Further studies are in progress.

Thus the activity of the present platinum pyrimidine greens against L1210 cells is comparable to that of cisplatin (*cis*-DDP) (IC_{50} = 2 ng/ml or 6.7 nM; *MIC* = 1.5 μg/ml measured in this work). These results may suggest homologous reaction mechanisms of biological activities. Note that for this family of compounds sample concentrations of 1–2 μM are sufficient to inhibit the cell growth entirely. Since the *MIC* value of *cis*-DDP is 5.0 μM, the present observation indicates that platinum pyrimidine greens have accumulation effects as oligomer complexes on the active site, probably nuclear DNA (*cf.* ref. 7). The higher activity of the smaller Pt uridine greens might be related to transport of the drug across the cell membrane. The low activity of UMP green and the high activity of uracil green, however, show that cytotoxic

activity cannot be explained only by cell membrane transport. The present results show that low substrate/Pt complexes could be promising chemotherapeutic agents, and that the one-pot synthesis opens the door to preparation of a wide variety of such materials.

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