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LETTER

HPLC Behavior and Cytotoxic Activity against Mouse Leukemia L1210 Cells of Platinum Pyrimidine Greens

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In a series of previous reports from this laboratory, we have shown that platinum pyrimidine greens are a potent growth inhibitor of mouse leukemia L1210 cells, whereas the corresponding blues are not [1–4]. Selective and efficient synthesis of platinum greens has been developed by hydrogen peroxide [5], photochemical [6] and one-pot [7] reactions, and characterization of Pt greens was carried out by NMR and ESR spectroscopy [8]. We have also discussed in an earlier paper the difference in activity of Pt greens prepared at 75° and 40 °C [4]. Consequently, we have examined in detail the relation between HPLC behavior of these Pt greens and their biological activity against L1210 cells *in vitro*, and herein report the results.

Experimental

cis-Diammineplatinum uridine greens were prepared according to the previously reported procedures via a one-pot reaction [7]. HPLC was performed with a TSK gel G2500PW$_{XL}$ column (Toso; 7.8 mm ID × 30 cm, monitored at 254 nm), and a mixed solution of 0.2 M H_2SO_4 and 0.2 M K_2SO_4 (pH 6) was used as an eluant at a 0.5 ml/min flow rate. The samples were dissolved in the deaerated buffer solution and kept at 4 °C in the dark for time-dependence experiments.

The antitumor activity of each sample was tested with leukemia L1210 cells in cell culture, and the inhibitory effect of the platinum complexes on the cell growth was examined. The number of cells and distributions of cell sizes were determined by a Coulter Counter ZM equipped with a Channelyzer 256. The cells between 6.4 and 16 μm in diameter were counted, and the antitumor activity is expressed as growth inhibition (*T/C%*) of the cells, which is the ratio of cell numbers for treated to untreated groups.

Results and Discussion

The molecular size of Pt-uridine greens was found to be smaller than that of the corresponding blues from their behavior on gel filtration with Toyopearl HW-40F [1, 5]. This was further confirmed in the present work by HPLC using Pt-α-pyrrolidone tan as an internal standard, the tetranuclear structure of which has been proved [9]. Namely, Pt-uridine blue, Pt-uridine green and Pt-α-pyrrolidone tan gave retention times, respectively, of around 15.3, 16–17.5 and 19.3 min at neutral pH, and therefore the size (volume) of the platinum green should be smaller than that of the corresponding blue, but obviously larger than that of Pt- α-pyrrolidone tan.

While the Pt blue gave a single peak at 15.3 min, the Pt greens prepared at 75° consisted of three peaks with retention times at 16.1 (Peak 1), 17.3 (Peak 2) and 18.0 min (Peak 3) in the HPLC chromatograms at pH 6. Similarly, the 40° sample showed three peaks under the same analytical conditions with slightly different retention times; i.e. at 15.6, 16.9 and 17.7 min, respectively, for Peak 1, Peak 2 and Peak 3.

Evidently ratios of the three peaks of the 75° sample were varied after 16 days in the dark at 4 °C, whereas no significant change was observed in both the highly active 40° sample and the inactive Pt-uridine blue.

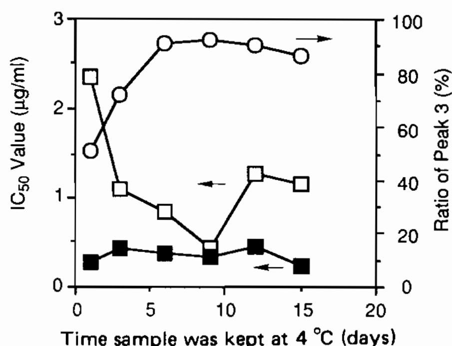

Fig. 1. Relations between time, and IC_{50} and Peak 3 ratio. Plots of activity against the time the sample was kept at 4 °C for the 75° sample (□) and 40° sample (■); plots of Peak 3 ratio in the 75° sample and time (○).

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TABLE 1. Peak ratios in HPLC[a] and IC_{50} values [b] for Pt-uridine greens

Run	Pt green[c]	Day[d]	Ratios (%)[e] of			IC_{50} (μg/ml)
			Peak 1	Peak 2	Peak 3	
1	Uridine at 75°	1	8.9	40.0	51.0	2.35
2		3	8.3	19.6	72.1	1.10
3		6	8.7		91.3	0.85
4		9	7.0		93.0	0.43
5		12	9.0		90.9	1.28
6		15	12.5		86.9	1.15
7	Uridine at 40°	1	12.5	23.6	63.4	0.25
8		3	14.8	23.8	60.5	0.35
9		6	15.4	22.1	62.2	0.33
10		9	15.4	20.7	63.1	0.26
11		12	12.4	17.6	69.9	0.36
12		15	12.3	23.5	64.0	0.20

[a] Toso TSK gel G2500PW_{XL} column (7.8 mm ID × 30 cm) eluted with a mixed solution of 0.2 M H_2SO_4 and 0.2 M K_2SO_4 at pH 6. [b]Concentration at 50% inhibition of cell growth. [c]Prepared at 75° for 45 min or 40° for 3 h according to the reported procedures (see ref. 7). [d]Kept in the dark at 4 ℃. [e]Peaks 1, 2 and 3 correspond to those with retention times around 16, 17 and 18 min, respectively; see text.

As shown in Fig. 1, plots of IC_{50} value and the time the sample was kept at 4 ℃ exhibit no inherent activity change for 2 weeks in the 40° sample (IC_{50} = 0.2–0.36, average value = 0.29 μg/ml), whereas the activity of the 75° sample apparently increases after 3 days (approximately twice).

These results strongly suggest structural variations of the 75° sample in solution and an increased ratio of the highly active species (IC_{50} = 0.43–2.35, i.e. five-fold activity differences over 15 days). Eventually, when a time dependence of the Peak 3 ratios in the 75° sample is observed (Fig. 1), there is considerable agreement on the whole between the activity and Peak 3. Namely, while the respective ratios of Peak 3 and Peak 2 on the first day are 51 and 40% (IC_{50} = 2.35 μg/ml), the former ratio clearly increases with a decrease of Peak 2 as time passes; viz., 72 and 19.6%, 91 and nil %, and 93 and nil % with a concomitant activity rise to give IC_{50} values of 1.10, 0.85 and 0.43 μg/ml, respectively, after 3, 6 and 9 days. Table 1 summarizes these results together with the cytotoxic activities against leukemia L1210 cells.

It is of interest that a remarkable enhancement of the activity after 9 days was always noticed in the 75° sample with the highest Peak 3 ratios from three independent experiments, and that the ratio decreased gradually with time. This phenomenon may be related to interconversions and an unusual thermochromic effect [3, 10] of the 75° sample in solution, but no further details are available at present. In the 40° sample, however, both the Peak 3 ratio and the biological activity were as a whole unaltered. While discrepancies in HPLC behavior of Peak 2 between the 40° and 75° samples suggest stability differences in the structures, details are unavailable at present. The most active compound may be contained in Peak 3, and efforts to obtain the fraction with highest activity are continuing.

Conclusions

The present study demonstrates that Pt-pyrimidine greens consist of three fractions with retention times around 16 (Peak 1), 17 (Peak 2) and 18 min (Peak 3) at pH 6 with a gel HPLC column (TSK gel G2500PW_{XL}) at a 0.5 ml/min flow rate, and that Peak 3 may be responsible for the growth inhibitory activity against L1210 cells. Isolation of Peak 3 and work on the biological mechanism [11] are currently under progress, and will be reported elsewhere.

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References

1 Y. Okuno, K. Tonosaki, T. Inoue, O. Yonemitsu and T. Sasaki, *Chem. Lett.*, (1986) 1947.
2 T. Shimura, T. Tomohiro, K. Maruno, Y. Fujimoto and Y. Okuno, *Chem. Pharm. Bull. Jpn.*, *35* (1987) 5028.

3 T. Tomohiro, T. Laitalainen, T. Shimura and Y. Okuno, in W. Ando and Y. Morooka (eds.), *The Role of Oxygen in Chemistry and Biochemistry*, Vol. 33, Elsevier, Amsterdam, 1988, pp. 557–562.
4 H(Y). Okuno, T. Shimura, T. Uemura, H. Nakanishi and T. Tomohiro, *Inorg. Chim. Acta, 157* (1989) 161.
5 T. Shimura, T. Tomohiro, T. Laitalainen, H. Moriyama, T. Uemura and Y. Okuno, *Chem. Pharm. Bull. Jpn., 36* (1988) 448.
6 Y. Okuno, T. Inoue, O. Yonemitsu, T. Tomohiro and T. Laitalainen, *Chem. Pharm. Bull. Jpn., 35* (1987) 3074.
7 T. Shimura, T. Tomohiro and H(Y). Okuno, *Inorg. Chim. Acta, 155* (1989) 21.
8 T. Uemura, T. Tomohiro, K. Hayamizu and Y. Okuno, *Chem. Phys. Lett., 142* (1987) 423.
9 K. Matsumoto and K. Fuwa, *J. Am. Chem. Soc., 104* (1982) 897.
10 T. Shimura, T. Okada, T. Tomohiro, M. Kodaka and H(Y). Okuno, *Abstr. 39th Symp. Coordination Chemistry of Japan, Mito, 1989*, p. 689 (3D14).
11 S. E. Serman and S. J. Lippard, *Chem. Rev., 87* (1987) 1153; J. P. Caradonna and S. J. Lippard, *Inorg. Chem., 27* (1988) 1454, and refs. therein.