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LETTER

Antitumor activity in association with thermochromic change of platinum pyrimidine greens against murine and human tumor cells

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Earlier work from this laboratory has shown that platinum pyrimidine greens are a potent growth inhibitor of mouse leukemia L1210 cells, whereas the corresponding blue materials are not [1–3]. A selective and efficient method for synthesizing platinum greens by facile one-pot reaction has therefore been established [3–5]. We have also demonstrated that there are differences in the cytotoxic activity of Pt greens depending on the preparation temperature, for instance, the sample synthesized at 40 °C exerted greater cytotoxic activity than that obtained at 75 °C [6, 7]. In this report, we examined the antitumor activity of Pt greens against various murine and human tumor cells. We also found that in all tumor cells examined the antitumor activity of the 75 °C sample varied in association with thermochromic change at the absorption maximum (722 nm), and that there was a considerable shift in size distribution of the tumor cells owing to the treatment with Pt greens.

Experimental

Materials

cis-Diammineplatinum green compounds with uridine were synthesized by the method previously reported as a one-pot reaction: sample 1 (S1) prepared at 40 °C; sample 2 (S2) prepared at 75 °C. The products were shown to be identical by elemental analysis and to have the unique green color of the Pt–uridine greens (λ_{max} = 265 and 722 nm in 10 mM H_2SO_4) [5, 7]. Sample 3 (S3): an aqueous solution of S2 was heated at 40 °C for 20 min, and then precipitated by the addition of acetone. Sample 4 (S4): S3 in water kept at 4 °C and isolated after 10 days in a similar manner as above.

Antitumor activity

The cytotoxic activity of each Pt green was evaluated *in vitro* with mouse leukemia (L1210), mouse sarcoma (S-180), human Burkitt lymphoma (Daudi), human epitheloid carcinoma (HeLa) and human histiocytic lymphoma (U937). L1210, Daudi and U937 were maintained with RPMI-1640 medium containing penicillin (100 unit/liter), streptomycin (100 mg/ml), 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 10 or 20% (for Daudi) fetal bovine serum (FCS). HeLa and S-180 were in MEM medium containing these same four materials (the FCS content was 10 and 5%, respectively) and 1% non-essential amino acids. Each tumor cell (1×10^5/ml) was cultured in the presence or absence of Pt greens for 48 h at 37 °C in 5% CO_2. Then the number and size distribution of the cells were assayed with a Coulter Channelyzer. Mitomycin C (10 μg/ml) was added to the medium instead of Pt green for a positive control. Activity was expressed as IC_{50} values defined as the concentration at 50% inhibition of cell growth unless otherwise noted.

Results and discussion

Antitumor activity associated with thermochromic change of Pt greens

All Pt greens used here showed significant cytotoxic activity towards all tumor cells employed (Table 1). The sensitivity of tumor cells to the Pt greens differed with S-180 being the most sensitive overall. However, we should emphasize the following three common facts observed in all five tumor cell lines: (i) the sample synthesized at 40 °C (S1) showed the highest activity among the Pt greens tested; (ii) when the 75 °C sample in 10 mM H_2SO_4 was warmed at 40 °C for 20 min (S3), the activity was remarkably reduced; (iii) this decreased activity returned to its original level if the solution was kept at 4 °C for more than 10 days (S4).

The above findings are of particular interest, since we have also observed an absorbance change at 722 nm associated with temperature. Intensity of the maximum in the visible region decreased conspicuously with a rise in temperature (Fig. 1(a)), for example absorbances at λ_{max} of S2 (5.6 mg/ml in 10 mM H_2SO_4) were 2.74, 1.81 and 0.16, respectively,

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TABLE 1. Cytotoxic activity and absorption intensity of Pt greens

Sample	IC_{50} (μg/ml)					A_{722}[a]	Temperature (°C)
	Murine cell		Human cell				
	L1210	S-180	Daudi	HeLa	U937		
S1	0.85	<0.05[b]	0.13	1.1	1.9	3.36	4
S2	2.9	0.11	2.2	2.4	4.9	2.74	4
S3	15	4.8	8.8	6.4	18	0.16	40
S4	2.9	<0.05[c]	2.3	3.5	5.0	2.74	4

[a]Absorbance at 722 nm in 10 mM H_2SO_4, 9.3 mg/ml for S1; 5.6 mg/ml for S2, S3 and S4. [b]cf. IC_{80}: 0.66. [c]cf. IC_{80}: 3.5.

at 4, 20 and 40 °C. However, no linear recovery of the intensity was observed with a fall in temperature; viz. return of the intensity was only 44% at 20 °C and 46% at 4 °C of the corresponding initial level (Fig. 1(b)). The time interval between the lines in Fig. 1 was approximately half an hour, therefore recovery of the absorbance seemed unusually slow in this instance. The intensity returned to the original level after 10 days at 4 °C as shown in Fig. 1(b) [8]. When, however, the sample was only warmed up to 30 °C, fast and linear recovery of the intensity was perceived (e.g. 103% restoration at 20 °C after warming S2 at 30 °C for 25 min). Thus this Pt green complex appears to follow a thermochromic change at least between 4 and 30 °C, but some drastic change in the molecular structure may have occurred at 40 °C under the conditions employed, which naturally reflects the antitumor activity. In fact, elemental analysis* revealed considerable ligand exchange between uridine and some S-containing ligand (possibly SO_4) in S2 and S3 complexes. Hence, lower analyses of C, H and N, and higher S content were observed in S3. Notably, when the solution was allowed to stand for 10 days at 4 °C under nitrogen, recurrence of the components (increase of C, H and N, and decrease of S) again resulted (S4), which was obviously associated with the absorbance change and the biological activity described above.

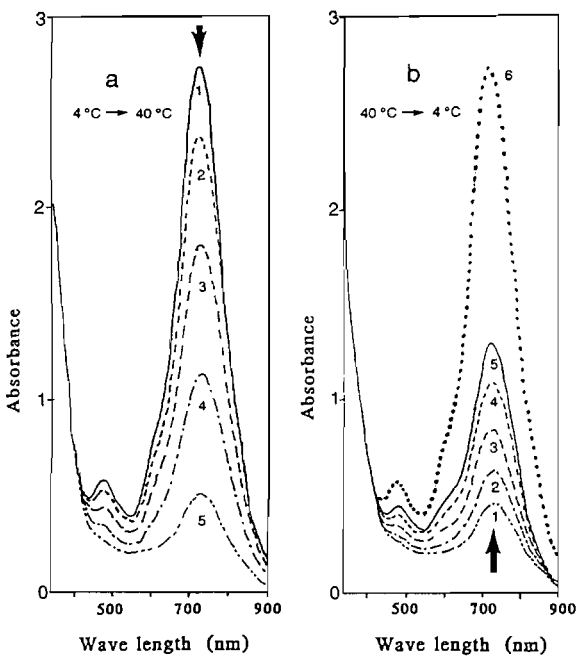

Fig. 1. Thermochromic change of visible region absorption. Platinum uridine green (S2; 5.6 mg/ml) in 10 mM H_2SO_4 at following temperatures: (a) with rising temperature: 1, 4; 2, 10; 3, 20; 4, 30; 5, 40 °C; (b) with lowering temperature: 1, 40; 2, 30; 3, 20; 4, 10; 5, 4 °C; 6, after 10 days at 4 °C under N_2. Each cycle took c. half an hour: approximately 5 min to reach the specified temperature and the sample was then kept at that temperature for 25 min.

These phenomena could be related to HPLC behavior and, in preliminary work we have to date obtained five green fractions (retention times: 170, 172, 183, 199 and 203 min) from S2 using Toyopearl HW-40 columns ((2×50 cm)×2) eluted with 1 mM H_2SO_4 (flow rate: 0.8 ml/min). Evaluation of the biological activity of each fraction with S-180 has manifested that the 199-min peak is extremely active (IC_{50} < 0.01 μg/ml), although all fraction show considerable activity (IC_{50}: 2.0–3.4 μg/ml for the other four). Therefore, it is probable that the ratio of the 199-min peak in molecules dominates the activity of Pt greens. Detailed work along this line is in progress and will be reported later in a full paper.

*Anal. S1: Found: C, 7.61; H, 2.84; N, 8.80; S, 6.96. Calc. for $[Pt_{10}(C_9H_{11}N_2O_6)_3(NH_3)_{20}(OH)_2(H_2O)_{14}](SO_4)_9$: C, 7.77; H, 2.97; N, 8.73; S. 6.92%. S2: Found: C, 15.31; H, 3.36; N, 8.89; S, 4.92. Calc. for $[Pt_{13}(C_9H_{11}N_2O_6)_{10}(NH_3)_{25}(H_2O)_9](SO_4)_{11}/22H_2O$: C, 15.42; H, 3.55; N, 8.99; S, 5.03%. S3: Found: C, 2.19; H, 0.87; N, 1.37; S, 15.18%. S4: Found: C, 14.33; H, 3.57; N, 8.44; S, 5.23%.

Size distribution of cells resulting from treatment with Pt greens

We further examined the size distribution of tumor cells after the treatment with Pt greens to determine the possible mechanism of biological activity. Figure 2 illustrates that the peak of cell distribution (L1210) became gradually larger according to the sample dose, and the antitumor activity was approximately parallel to the dosage amount. We previously reported that macrocells which were roughly twofold larger in diameter than normal L1210 cells emerged in effective samples and that the more effective the sample was, the denser was the population of these cells [2]. This change in size distribution was observed in all tumor cell lines examined here by the treatment with all Pt greens. We observed that the more effective the sample was, the larger was the shift.

It was reported that in human fibroblasts when replication rates were slowed by low temperature or low serum concentration or by inhibition of DNA synthesis, a clear shift to larger cells occurred [9]. The same report suggested that the increase in volume of cells with impaired replicative ability was the result of a primary abnormality in cell replication. Therefore, we also assumed that our sample of Pt greens acted as a replication inhibitor, probably because of the interaction with DNA. The present observation on cell distribution enlargements thus strongly supports the activity of the samples as a replication inhibitor. Further investigation on the mechanism of the antitumor Pt greens is currently underway including its effect on cell cycles.

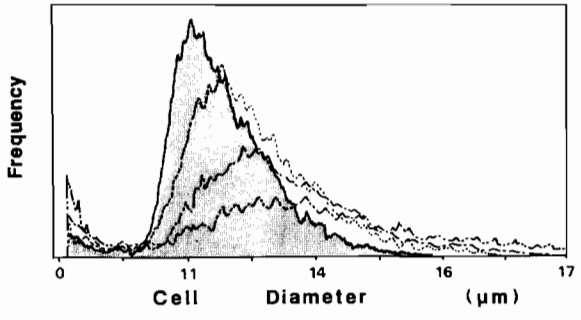

Fig. 2. Size distribution of the tumor cells after treatment with Pt greens. L1210 (1×10^5/ml) was treated with Pt green (S3) for 48 h, and the distribution of cell size was examined with a Coulter Channelyzer. Doses (mg/ml): ——, 1.25; – – –, 2.5; – – –, 5.0; – – – –, 10.0.

Conclusions

We have demonstrated that Pt greens show outstanding cytotoxic activity towards a variety of murine and human tumor cells such as L1210, S-180, Daudi, HeLa and U937, and that a remarkably active fraction could be identified from HPLC analysis with a gel column. The activity is associated with thermochromic change of the green materials. In addition, examinations of size distribution of cells have suggested that Pt greens act as a replication inhibitor.

Acknowledgements

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