Antitumor Activity of New Antitumor Substance, Polyoxomolybdate, against Several Human Cancers in Athymic Nude Mice

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—— Antitumor polyoxomolybdates have been recognized in the course of study on the medical utilization of polyoxometalates, inorganic polymers of metal oxide. $[\text{NH}_3\text{Pri}]_6[\text{Mo}_7\text{O}_{24}]\cdot 3\text{H}_2\text{O}$ (PM-8) was found as a representative of antitumor polyoxomolybdates. The growth suppressions of PM-8 against Co-4 human colon cancer xenografted under the subrenal capsule in cd-1 mice were equal or superior to that of 5-FU, MMC, ACNU, ADM and CDDP. Potent antitumor activity of PM-8 is also established against MX-1 human breast and OAT human lung cancer xenografted in athymic nude mice. Polyoxomolybdate is a new type of antitumor substance. ——— antitumor effect; polyoxomolybdate; human tumor; xenograft; nude mouse

The use of cis-diamminedichloroplatinum (CDDP) has been now been proved to be significant in treating mouse tumors and several human tumors, particularly the seminomas (Rosenberg et al. 1969). Dramatic improvement have been made in the treatment of patients with disseminated testicular tumors by modern chemotherapeutic protocols, which are based on the dose-dependent antitumor activity of CDDP (Samson et al. 1984). However, the excellent antitumor activity of CDDP is accompanied with strong toxic effects. From chemical analogy with CDDP, the antitumor active compounds such as titanocene dichloride and copper (I), silver (I), and gold (I or III) tetrahedral diphosphine complexes have been investigated (Harstrick et al. 1983). In addition, organosilicone compounds of other organometallic complexes with favorable antitumor properties, such as trimethylsilylethylthioethylamine and its derivatives, have been found (Toyoshima et al. 1985; Fujita et al. 1990, Sakurai 1990). In the course of our examination of biological effects of polyoxometalates, inorganic polymers of metal

oxide, significant antitumor effect of polyoxomolybdates was found against conventional murine tumors (Yamase et al. 1988). This paper describes additional potent activities of the polymolybdates against human cancer xenografts, as a way of realizing the chemical usage of these substances.

MATERIALS AND METHODS

Three human tumors were used throughout this study; Co-4 colon cancer, MX-1 breast cancer and OAT lung cancer. We used Balb/c athymic nude mice for antitumor evaluation by implantation method and female cd-1 mice for subrenal capsule assay (SRC assay).

RESULTS

Antitumor evaluation against Co-4 by SRC assay

From the screening of fifty polyoxometalates for antitumor activities using SRC assay, antitumor activities were found for four polyoxomolybdates of PM-8, 17, 26 and 32. The activities of the polyoxomolybdates were compared with those of the reference antitumor drugs in Table 1. Both PM-8 and PM-17 showed significant activities, however, the latter exhibited a strong toxicity as represented by a severe body weight loss in the mice.

Antitumor activities of PM-8 against MX-1 and OAT by SRC assay

The activities of PM-8 against MX-1 and OAT were compared with those of

TABLE 1. *Antitumor activity of polyoxometalates against Co-4 human cancer xenograft under the subrenal capsule in mice*¹

Compound ²	Dose (mg/kg)	Treatment schedule	Relative body weight (day 6/day 0)	Relative tumor size (day 6/day 0)
Tumor control	Vehicle	day 1-day 5 (i.p.)	1.03±0.01 ³	1.51±0.11 (—) ⁴
PM-8	200	day 1-day 5 (i.p.)	1.07±0.01	0.67±0.09 (54.3) ^c
PM-17	25	day 1-day 5 (i.p.)	0.70±0.05	0.87±0.06 (42.2) ^c
PM-26	50	day 1-day 5 (i.p.)	1.02±0.02	1.11±0.05 (26.5) ^a
PM-32	100	day 1-day 5 (i.p.)	1.03±0.01	1.06±0.04 (29.7) ^b
CDDP	2	day 1-day 5 (i.p.)	0.97±0.03	0.82±0.03 (46.6) ^a
5-FU	30	days 1, 4 (i.p.)	0.99±0.01	0.85±0.02 (44.0) ^a
MMC	2	days 1, 4 (i.p.)	1.04±0.01	0.83±0.02 (44.9) ^a
ACNU	10	days 1, 4 (i.p.)	1.01±0.01	0.94±0.09 (37.6) ^b
ADM	5	days 1, 4 (i.p.)	0.97±0.02	0.87±0.02 (42.2) ^a

¹At day 6 after tumor implantation, tumor sizes of mice treated with chemicals were compared to that of vehicle control mice. ²PM-8, [NH₃Pri]₆[Mo₇O₂₄]₃·3H₂O; PM-17, [NH₃Pri]₆[H_xMo₇O₂₄] (x=1–2); PM-26, [NH₃Pri]₆[Mo₇O₂₆]₃·3H₂O; PM-32, Na₆[IMo₆O₂₄]₃·34H₂O. ³Mean±s.e. of five mice. ⁴Values in parentheses indicate % inhibition of tumor growth. a, b and c indicate significant differences in the values for relative tumor size between vehicle control mice and treated mice ($p < 0.02$, $p < 0.01$ and $p < 0.001$, respectively).

TABLE 2. *Antitumor effect of PM-8 against human cancer xenografts implanted under the subrenal capsule in mice*¹

Tumor	Compound	Dose (mg/kg)	Treatment schedule	Relative tumor size (day 6/day 0)
MX-1	Tumor control	Vehicle	day 1-day 5 (i.p.)	1.45 ± 0.15 ²
	PM-8	200	day 1-day 5 (i.p.)	0.75 ± 0.14 ^a
		100	day 1-day 5 (i.p.)	0.80 ± 0.07 ^b
	5-FU	30	day 1-day 5 (i.p.)	0.81 ± 0.10 ^a
OAT	Tumor control	Vehicle	day 1-day 5 (i.p.)	2.45 ± 0.25
	PM-8	200	day 1-day 5 (i.p.)	1.40 ± 0.07 ^a
		100	day 1-day (i.p.)	1.55 ± 0.15 ^a
	5-FU	30	day 1-day (i.p.)	0.65 ± 0.05 ^c

¹At day 6 after tumor implantation, tumor sizes of mice treated compound were compared to that of vehicle control mice. ²Mean ± s.e. of five mice. a, b and c indicate significant differences between saline control group and treated groups ($p < 0.05$, $p < 0.02$ and $p < 0.001$, respectively).

5-FU. The doses were 200 mg/kg and 100 mg/kg for PM-8 and 30 mg/kg for 5-FU. PM-8 exhibited significant antitumor activities against both human cancer xenografts shown in Table 2. Against MX-1, the activity of PM-8 was equal to that of 5-FU, which showed a T/C value of 51% ($p < 0.05$), 54% ($p < 0.02$) at 200 mg/kg and 100 mg/kg, respectively, and 5-FU displayed a T/C value of 56% ($p < 0.05$). On the other hand, against OAT, 5-FU showed significant activity with a T/C value of 9.6% ($p < 0.001$). The T/C values of PM-8 were 61.9% ($p < 0.05$) at 200 mg/kg and 71.6% ($p < 0.05$) at 100 mg/kg.

TABLE 3. *Antitumor effect of PM-8 against human cancer xenografts in nude mice*¹

Tumor	Compound	Dose (mg/kg)	Treatment schedule	% of inhibition of tumor growth at		
				day 10	day 20	day 30
MX-1	Tumor control	Vehicle	day 1-day 10 (i.p.)	—	—	—
	PM-8	200	day 1-day 10 (i.p.)	60 ^b	62 ^b	70 ^d
		100	day 1-day 10 (i.p.)	50 ^a	40 ^a	62 ^d
	5-FU	30	days 1, 3, 5, 7, 9 (i.p.)	80 ^c	80 ^d	82 ^d
OAT	Tumor control	Vehicle	day 1-day 10 (i.p.)	—	—	—
	PM-8	200	day 1-day 10 (i.p.)	70 ^a	69 ^d	63 ^d
		100	day 1-day 10 (i.p.)	40	50 ^a	54 ^d
	5-FU	30	days 1, 3, 5, 7, 9 (i.p.)	85 ^c	77 ^c	75 ^d

¹Mice were used in groups of five. a, b, c and d indicate significantly differences between saline control group and treated groups ($p < 0.05$, $p < 0.02$, $p < 0.01$, $p < 0.001$, respectively).

Antitumor activities of PM-8 against MX-1 and OAT in nude mice

The antitumor activities of PM-8 against xenografts implanted in nude mice were investigated. The experimental result was shown in Table 3. PM-8 showed significant antitumor activities against MX-1 and/or OAT human cancer in nude mice.

DISCUSSION

The antitumor activities of the polyoxometalates were evaluated using a human tumor-nude mouse system and compared with those of antitumor chemotherapeutic drugs. Of polyoxometalates with their structures based on closely packed oxygen arrays containing interstitial metal centers, PM-8, 17, 26 and 32 were found to exhibit antitumor activities against Co-4 human colon cancer xenograft using SRC assay in mice. Especially, PM-8 was the most active. The geometry of $[\text{Mo}_7\text{O}_{24}]^{6-}$ for PM-8, consisting of seven MoO_6 octahedra condensed by edgesharing into the structure is shown in Fig. 1.

It is notable that PM-8 showed antitumor activities against human cancer xenografts. The conventional murine tumor systems have contributed greatly to the development of new antitumor agents, but they are not necessarily clinically effective. Many active compounds in these systems have been clinically inactive. On the other hand, the human tumor-nude mouse systems have been more useful for identifying new anticancer agents of clinical interest.

Particularly, it has been reported that the effects of 5-FU, ADR or CDDP against several human tumor xenografts reflect the clinical response rate very closely. They are the most potent antitumor drugs currently available, but their activities are not necessarily sufficient in terms of curative therapy for cancer patients. So more active antitumor agents are still needed.

Fig. 1. Structure of $[\text{Mo}_7\text{O}_{24}]^{6-}$. ○, oxygen atom; ●, Mo atom.

Since PM-8 exhibited significant antitumor activities in human cancer-nude mouse systems, it could be a promising candidate for clinical studies. To study the structure-activity relationship of PM-8, the influence of chemical variation on the murine tumor inhibiting activity was investigated. This modification was made in three different ways: (1) the $[\text{NH}_3\text{Pri}]^+$ cation was replaced by $[\text{NH}_4]^+$ and K^+ ; (2) the $[\text{Mo}_7\text{O}_{24}]^{6-}$ anion was replaced by Cl^- ; (3) the d^0 configuration of a Mo atom in $[\text{Mo}_7\text{O}_{24}]^{6-}$ was changed photochemically to the d^1 configuration $[\text{H}_x\text{Mo}_7\text{O}_{24}]^{6-}$.

Antitumor activities of these modified compounds against Meth A sarcoma were investigated. $[\text{NH}_4]^{6-} [\text{Mo}_7\text{O}_{24}] \cdot 4\text{H}_2\text{O}$ and $\text{K}_6[\text{Mo}_7\text{O}_{24}] \cdot 4\text{H}_2\text{O}$ were effective, whereas $[\text{NH}_3\text{Pri}]\text{Cl}$ was ineffective against Meth-A tumor bearing mice. Especially, $[\text{NH}_3\text{Pri}]^{6-} [\text{H}_x\text{Mo}_7\text{O}_{24}]$ exhibited a significant inhibition on the growth of Meth A sarcoma. The results indicate that the polyoxomolybdate structure of the Mo_7O_{24} is apparently of critical significance for the antitumor activity. The administration of PM-17 caused a severe body weight loss, suggesting that the reduction of PM-8 by up to two electrons results in an adverse effect on the mice as seen in Table 1. The action mechanism on antitumor activity of PM-8 is not yet clear. PM-8 exhibited non specific weak interaction with DNA (Tomita et al. 1989). Thus PM-8 can be distinguished from the antitumor mechanism of a mononuclear metal complex such as CDDP and other organometallic compounds.

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