Medical Chemistry of Polyoxometalates. Part 1. Potent Antitumor Activity of Polyoxomolybdates on Animal Transplantable Tumors and Human Cancer Xenograft

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The use of *cis*-diamminedichloroplatinum(II) (cisplatin) has now been proved to be significant in treating several human tumors, particularly the seminomas [1, 2]. From chemical analogy with cisplatin, the antitumor-active compounds such as titanocene dichloride and copper(I), silver(I), and gold(I or III) tetrahedral diphosphine complexes have been investigated. Preliminary results from these compounds indicate that the anticancer mechanism is likely to be different from that of square-planar cisplatin which inhibits DNA as a template for replication [2, 3]. In addition, organosilicon compounds of other organometallic complexes with favorable antitumor properties, such as trimethylsilylethylthioethylamine and its derivatives, have been found [4].

On the other hand, in the course of studies on the photoredox chemistry of polyoxometalates with wide varieties in structure, ionic size and charge, solubility in water, and multi-electron redox properties [5], we have tried to apply the photoredox chemistry of polyoxometalates to biomedical fields. This paper describes heptamolybdates as a new type of antitumor substance, especially hexakis(isopropylammonium) heptamolybdate(VI) trihydrate $[NH_3Pr^i]_6[Mo_7O_{24}]\cdot 3H_2O$ (PM-8) which inhibits 80% of the tumor growth in mice bearing methylcholanthrene-induced tumor (Meth A sarcoma) and MM-46 adenocarcinoma. Moreover, PM-8 exhibits significant anticancer activity (arrest of tumor growth of 73% of the control after 19 days of treatment) against human breast carcinoma implanted in nude mice. The growth suppression is superior to that obtained with 5-fluorouracil (5-FU) and 1-(4-amino-2-methylpyrimidin-5-yl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU), which are clinically approved drugs showing good activity against human colon carcinomas. Although there are few papers on the biological properties of polyoxometalates [6], 21-tungsto-9-antimonate $[Sb_9W_{21}O_{86}]^{19-}$ has recently been investigated as an acquired immunodeficiency syndrome (AIDS) antiviral drug, inhibiting the reverse transcriptase of both lymphocytopathic retrovirus and simian AIDS virus [7].

Experimental

$[NH_3Pr^i]_6[Mo_7O_{24}]\cdot 3H_2O$ (PM-8) and its reduction species were synthesized according to previous procedures [8]. The reduced form of PM-8 was prepared by UV ($\lambda > 250$ nm) photolysis of PM-8 (13.6 mM) in aqueous solution. A brown product was precipitated as the $[NH_3Pr^i]^+$ salt when the deaerated photolyte obtained through a prolonged photolysis was condensed below 50 °C, and separated by filtration. Analysis of Mo^V in the brown powder by titration with $KMnO_4$ indicated a mixture of one- and two-electron reduction species of PM-8. The absorption spectrum ($\lambda_{max} = 510$ nm) of the brown powder was similar to that of the UV-irradiated PM-8 solid, implying that there is little difference in the anion structure between the photoreduced precipitate and PM-8 [8, 9]. Considering the fact that the photoreduction of polyoxometalates accompanies protonations [8, 10], the photoreduction product was assumed to have the composition $[NH_3Pr^i]_6[H_xMo_7O_{24}]$ ($x = 1 \sim 2$) (PM-17) where the number of lattice water molecules was not known. $K_6[Mo_7O_{24}]\cdot 4H_2O$ was synthesized according to the reported literature procedure [11]. All other reagents were at least analytical grade and were used as supplied.

Female BALB/c and C3H/He mice, with an initial body weight of 20 to 22 g, were housed in plastic cages under standard laboratory conditions with free access to food and water. BALB/c mice were implanted subcutaneously (s.c.) or intraperitoneally (i.p.) with 1.0×10^5 of Meth A sarcoma cells/mouse on day 0. C3H/He mice were implanted s.c. with 5.0×10^5 of MM-46 adenocarcinoma cells per mouse on day 0. Various dosages of PM-8 were administered i.p. for 9 consecutive days starting on day 1. To pursue the progress of the disease the mice were weighed daily. The 2×2 mm grafts of MX-1 human breast cancers were sterilely implanted into 6-week-old female BALB/c nude mice (nu/nu) through a flank incision midway between front and hind legs.

The mean survival time for the tumors was measured in days, and from this the increase in life-span (ILS) was calculated according to the equation % ILS = $100(t - c)/c$, where t is the mean survival time of the treated group and c is the mean survival time of the control group. In all tumor systems > 25% ILS

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was considered necessary to demonstrate activity. The antitumor activities were determined from the tumor-weight-inhibition (TWI) on specific days (as shown in the Tables) after tumor transplantation. Tumor weights (mg) were estimated by measuring the length (l) and width (w) of each tumor with a vernier caliper (mm) and using the formula $lw^2/2$.

Results and Discussion

Tables I and II demonstrate PM-8 to be a potent antitumor agent against Meth A sarcoma and MM-46 adenocarcinoma. PM-8 was administered i.p. daily on days 1 to 9 after subcutaneous or intraperitoneal implantation of tumor cells into the mice (8 to 11

TABLE I. Antitumor Effect of PM-8 Against Meth A Sarcoma and MM-46 Adenocarcinoma

Experiment number	Route to tumor implantation	Compound	Dose i.p. x 9 (mg/kg/day)	Body weight change (g) on day 14	TWI (%) on day 14	ILS (%)
Meth A sarcoma						
1	s.c.	Tumor control		(+)3.2		
		PM-8	100	(+)2.5	83**** [a]	63****
			50	(+)1.7	38****	32***
		ACNU	5	(+)1.6	57****	38****
2	s.c.	Tumor control		(3)2.5		
		PM-8	200	(+)2.2	64****	61****
		5-FU	68[b]	(+)0.4	52***	37**
3	s.c.	Tumor control		(+)2.6		
		PM-8	250	(+)0.8	44*	69****
		5-FU	20	(−)2.5	80****	19
		ACNU	10	(−)1.1	99****	47****
MM-46 Adenocarcinoma						
4	s.c.	Tumor control		(+)2.4		
		PM-8	200	(+)1.8	58****	111****
			100	(+)2.0	80****	167****
			50	(+)1.8	59****	121****

[a]Significantly different from corresponding tumor control group (*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$). [b]5-FU was administered orally on days 1, 5 and 9.

TABLE II. Antitumor Effects of PM-8 Derivatives against Meth A Sarcoma

Experiment number	Route to tumor implantation	Compound	Dose i.p x 9 (mg/kg/day)	Body weight change (g) on day 10	TWI (%) on day 14	ILS (%)
1	s.c.	Tumor control		(+)2.8		
		$[NH_3Pr^i]Cl$	100	(+)1.7	14	19
		$[NH_4]_6[Mo_7O_{24}]\cdot 4H_2O$	100	(+)1.2	31**** [a]	33****
		$K_6[Mo_7O_{24}]\cdot 4H_2O$	100	(+)0.2	50****	58****
		PM-8	50	(+)1.7	38****	32****
		PM-17	25	(−)2.2	45****	34****
2	i.p.	Tumor control		(+)5.6		
		PM-8	200	(+)4.3		44***
			100	(+)3.9		48***
			50	(+)2.0		111****
		PM-17	100[b] (i.p. x 3)	(−)2.2		127****
			50 (i.p. x 7)	(−)4.9		216**** [c]

[a]Significantly different from corresponding tumor control group (***$P < 0.01$, ****$P < 0.001$). [b]Two of 11 mice per group died on days 4 and 8 after injection of the compound. [c]Two of 11 mice per group survived over 60 days after tumor implantation and two mice were free from the tumor.

mice/group) on day 0. One daily administration of 100 mg/kg of PM-8 starting on day 1 produced a significant inhibition of Meth A sarcoma (ILS, 63%) for s.c. implants, as shown in Table I. Similarly, PM-8 administered i.p. at a dose of 50 mg/kg produced a remarkable prolongation of life-span (ILS, 111%) for i.p. implants, as shown in Table II. High values of ILS for PM-8 compared with 5-FU and ACNU (which are well known as approved drugs showing good activities against Meth A sarcoma) indicate that the antitumor activity of PM-8 is superior to 5-FU and ACNU. The administration of high doses of 5-FU and ACNU led to toxic deaths of mice, as was suggested by negative changes in body weight (expt. No. 3 in Table I). PM-8 was also strongly effective against s.c. implants of MM-46 adenocarcinoma: one daily administration (days 1 to 9, i.p.) of 100 mg/kg of PM-8 after the subcutaneous implantation of the MM-46 cells into the mice (8 mice/group) on day 0 produced 80% TWI and 167% ILS. The data in the Tables show that a dose effect on the inhibition of growth of both Meth A sarcoma and MM-46 adenocarcinoma is not clearly seen. In spite of the schedule of the high dose of PM-8 injected (250 mg/kg), no apparent toxic effect was noticed in the mice, since the mice used in these experiments maintained, on average, their weight throughout the 14 days. This excludes the possibility that the tumor growth inhibition is due to toxic effects on the host.

The effectiveness of PM-8 against animal transplantable tumors led us to expect an inhibition of the progressive growth of small xenografts of human neoplasms. The experiment was carried out by treating MX-1 human breast cancer xenografts in athymic nude mice. The results are shown in Fig. 1. Growth of MX-1 human breast cancers implanted s.c. in all nude mice (4 mice/group) proceeded unchecked after their implantation on day 0. Usually tumor growth was detected at the end of 17 days when tumor sizes ranged from average values of $350 \sim 491$ mm^3. Starting at this date it was usually easy to measure the three diameters to be averaged to obtain the tumor volume determinations. The first injection of PM-8 was made on day 17. Ten administrations (day 17 to 27, except 19, i.p.) of 200 mg/kg of PM-8 achieved a tumor growth inhibition (73%) such that on day 46 the breast tumor was 27% the size of that (7466 mm^3) of the tumor control group, without any special risk to the mice as long as they were appropriately sterilized by filtration. The reduced tumor size results from a much slower increase in breast cancer growth in the PM-8-treated mice *versus* the control, showing the potent activity of PM-8 to inhibit the growth of human breast cancers.

To study the structure–activity relationship of PM-8, the influence of chemical variation upon the Meth A sarcoma-inhibiting activity was investigated.

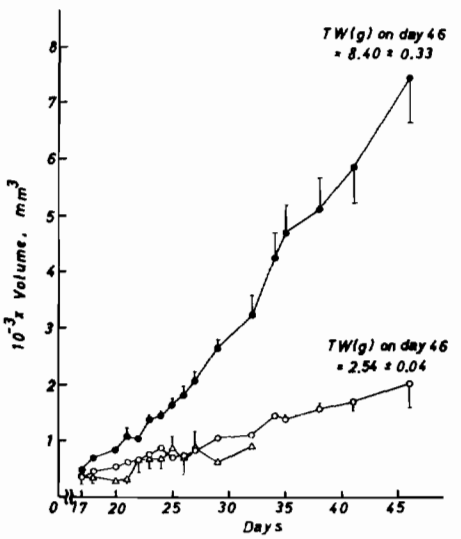

Fig. 1. Significance of the $[Mo_7O_{24}]^{6-}$ framework for anticancer activity. Tumor volume increases after implantation on day 0 and follow-up to 46 days, of MX-1 human breast cancers grown in 6-week-old female BALB/c nude mice, which were given injections of nothing (control, –●–), PM-8 (200 mg/kg/day, on days 17 to 27, except 19, i.p., –○–), and PM-17 (100 mg/kg/day, on days 17, 18, 20 and 25 mg/kg/day, on days 21 to 27, i.p., –△–). Points, means of four mice: bars, standard error. The treatment of PM-17 exhibited −5.0 body weight change (g) on days 17 to 32, whereas PM-8 resulted in no significant change on days 17 to 46.

This modification was made in three different ways: (i) the $[NH_3Pr^i]^+$ cation was replaced by $[NH_4]^+$ and K^+; (ii) the $[Mo_7O_{24}]^{6-}$ anion was replaced by Cl^-; (iii) the d^0 configuration of a Mo atom in $[Mo_7O_{24}]^{6-}$ was changed photochemically to the d^1 configuration in which the d^1 electron is almost localized due to a small degree of hopping into neighboring $Mo^{VI}O_6$ sites [8, 11]. Sites for the photoreduction of the $Mo^{VI}O_6$ to $Mo^{V}O_5(OH)$ in $[Mo_7O_{24}]^{6-}$ belong to two ends (two-electron reduction) or one at alternate ends (one-electron reduction) of three MoO_6 octahedra in a line in the central horizontal level of the $[Mo_7O_{24}]^{6-}$ framework [9, 12], as is shown in Fig. 2. Antitumor activities of these modified compounds against Meth A sarcoma are summarized in Table II. $[NH_3Pr^i]Cl$ (100 mg/kg) was ineffective against s.c. implanted Meth A sarcoma, whereas $[NH_4]_6[Mo_7O_{24}]\cdot 4H_2O$ and $K_6[Mo_7O_{24}]\cdot 4H_2O$ were effective. One daily administration of 25 mg/kg (days 1 to 9, i.p.) of PM-17 produced a significant inhibition of Meth A sarcoma on day 14 (45% TWI) and one daily dose of 50 mg/kg (days 1 to 7, i.p.) produced 216% ILS. The results indicate that the polyoxomolybdate structure of the Mo_7O_{24} framework (Fig. 2) is apparently of critical significance for the antitumor action. Furthermore, every administration of PM-17 induced a negative change in the body

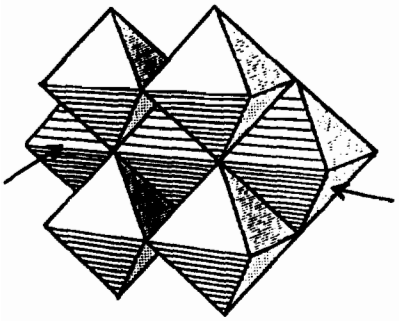

Fig. 2. Structure of $[Mo_7O_{24}]^{6-}$ and photoreducible MoO_6 sites (shown by arrow).

weight on day 10, suggesting that the reduction of PM-8 by up to two electrons results in a toxic effect on the host. The apparent signs of toxicity of PM-17 were observed for the nude mice (4 mice/group) grafted with the MX-1 human breast cancers (Fig. 1): three administrations (days 17, 18 and 20 i.p.) of 100 mg/kg of PM-17 after the implantation of the MX-1 xenografts on day 0 produced a death of one mouse on day 22; one daily administration (days 21 to 27, i.p.) of 25 mg/kg resulted in toxic deaths of another mouse on day 29 and others on day 34. Simultaneously, the therapy gave a tumor-growth inhibition showing 25% (673 mm^3) of the tumor size of the tumor control group on day 29. Thus, it is possible to say that in PM-17 the reduction of PM-8 by up to two electrons causes similar cancerostatic potency and strongly toxic phenomena.

The fact that the d^1 configuration of a Mo site in the Mo_7O_{24} framework exhibits strong toxicity is in contrast with the d^0 configuration, since the d^0 configuration of all Mo sites in PM-8 looks promising in toxicity: in spite of the increased amount of PM-8 injected (200 mg/kg day injection, i.p., 2.0 g/kg total) no apparent toxicity was noticed in the nude mice. The reduction of PM-8 to PM-17 occurred under the photoexcitation of the oxygen-to-molybdenum charge-transfer (LMCT) band in PM-8 [8, 9]. Therefore, a significant difference in toxicity between PM-8 (d^0 configuration) and its reduction species (d^1 configuration) provides a clue to the mechanism of the antitumor potency of the $[Mo_7O_{24}]^{6-}$ framework, assuming that the molecular interaction between $[Mo_7O_{24}]^{6-}$ and tumor cells involves thermal activation of the O → Mo LMCT band in a similar manner to the photoreaction. In other words, the reduction of $[Mo_7O_{24}]^{6-}$ to $[H_xMo_7O_{24}]^{6-}$ in the tumor cells, leading to the specificity of tumor cell killing by $[H_xMo_7O_{24}]^{6-}$, seems to reflect the antitumor potency of $[Mo_7O_{24}]^{6-}$. The strong toxicity of the d^1 configuration in the Mo_7O_{24} framework may be explained by the reduction of the host cells due to the highly negative oxidation potential ($\approx -0.10-0.06 \times pH$, -0.52 V *versus* SCE at pH 7 [13]) of $[H_xMo_7O_{24}]^{6-}$. The plausible redox reaction between $[H_xMo_7O_{24}]^{6-}$ and host cells results in $[Mo_7O_{24}]^{6-}$ formation and cytotoxicity (due to the reduction of the host cells). $[Mo_7O_{24}]^{6-}$ produced in the host cells will be transmitted to the tumor cells and contribute to the tumor cell killing due to the reproduction of $[H_xMo_7O_{24}]^{6-}$ as a result of the redox reaction with the tumor cells.

The variation of the cations in the Mo_7O_{24} model system may influence the residence time of $[Mo_7O_{24}]^{6-}$ in the tumor cells in addition to changes in its solubility in water. Our proposal that the anticancer activity of polyoxomolybdates is based on a reversible redox reaction of $[Mo_7O_{24}]^{6-} + e^- + H^+ \rightleftharpoons [HMo_7O_{24}]^{6-}$ implies that the antitumor mechanism of PM-8 is different from that of mononuclear metal complexes such as cisplatin and other organometallic compounds, since the mononuclear complexes form chelating bonds to biological macromolecules after dissociation of the chloride ligands [2, 3]. Furthermore, we remark that $(C_5H_5)_2MCl_2$ ($M = V^{IV}$, Nb^{IV} and Mo^{IV}) with d^1 and d^2 configurations for metallocene dichlorides increased the cytotoxicity, compared to a d^0 configuration as for $(C_5H_5)_2TiCl_2$ [14]. Further studies to elucidate details of the antitumor action of polyoxomolybdates are in progress.

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