A NEW TYPE OF ANTITUMOR SUBSTANCES, POLYOXOMOLYBDATES

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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. $[NH_3Pr^i]_6$ $[Mo_7O_{24}]\cdot 3H_2O$ (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 renal 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 was also established against MX-1, human breast cancer, and OAT, human lung cancer, xenografted in athymic nude mice. Polymolybdate is a new type of antitumor substance.

Key words: antitumor substance, polyoxometalate, polyoxomolybdate

INTRODUCTION

The use of cis-diamminedichloroplatinum (CDDP) has now been proved to be significant in treating mouse tumors and several human tumors, particularly the seminomas[1,2]. Dramatic improvements 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[3,4]. Unfortunately, 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 inventigated[2,5]. In addition, organosilicone compounds of other organometallic complexs with favorable antitumor properties, such as trimethylsilylthioethylamine and its derivatives, have been founds[6~8] In the course of our examination of biological effects of polyoxometalates, inorganic polymers of metal oxide, significant antitumoral effect of polyoxomolybdates was found against conventional murine tumors[9]. 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

Animals: We used female athymic nude mice (nu/nu) with a Balb/c genetic back-ground (CLEAR Japan, Tokyo) and female cd-1 mice (Charlus River Japan, Atugi). At the start of the experiments the mice were 5 to 6 weeks old and they were kept under specific pathogen-free conditions in an isolator.

Tumors: Three human tumors were used throughout this study. Co-4 (colon cancer) and MX-1 (breast cancer) were kindly donated by Dr. Abe (Dept. of Surgery, School of Medicine, Keio Univ.). OAT (lung cancer) was kindly provided by Dr. Ohboshi (Dept. of pathology, Niigata Univ.).

These tumors were maintained by passage in Balb/c athymic nude mice in our laboratory.

Chemicals: $[NH_3Pr^i]_6$ $[Mo_7O_{24}]\cdot 3H_2O$ (PM-8), $[NH_3Pr^i]_6$ $[H_xMo_7O_{24}]$ $(x=1-2)$ (PM-17)[10~12] and $Na_5[IMo_6O_{24}]\cdot 34H_2O$ (PM-32)[13] have been prepared, purified and characterized as previously described. $[NH_3Pr^i]_6$ $[Mo_7O_{26}]\cdot 3H_2O$ (PM-26) was obtained by the modification of the Stomberg's rethod[14] and its substance was characterized by a single crystal x-ray crystallography.

Antitumoral drugs: Antitumoral drugs used as the standard reference were as follows 5-FU; 5-Fluorouracil, MMC; Mitomycin C, ACNU; 1-(4-amino-2-methylpyrimidine-5-yl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride, ADM; Adriamycin, CDDP; cis-platinum diammine dichloride.

Antitumor activity test:

1) The first screening was performed by a modification of Bogden's method[15]. Co-4 solid tumors were minced into 2-mm cubed fragments and immediately placed in McCoy's medium. Then a single fragment was implanted on day 0 under the renal capsule of cd-1 mice under anesthesia.

The initial tumor size was recorded as the mean of 2 perpendicular diameters [$T_0 = (L+W)/2$] which were measured under a stereomicroscope equipped with an ocular micrometer. The mice were given the compounds i.p. according to the treatment schedule shown in Table 1. On day 6, the animals were killed by cervical dislocation, and the final tumor size (T_6) was measured. The change in tumor size over 6 days was computed for each xenograft as the relative tumor size (T_6/T_0).

2) Therapeutical treatments were used as follows. Solid tumors (MX-1 and OAT) produced on the backs of mice were measured every other day with a vernier caliper. For each tumor, the 2 perpendicular principle diameters were recorded, and the estimated tumor volume was expressed as $V = 1/2 \times \text{length (mm)} \times [\text{width (mm)}]^2$. When the tumor volume exceeded 100 mm^3, the treatment with a compound or a drug was started. The doses were 100 mg/kg/day or 200 mg/kg/day for PM-8 and 30 mg/kg/day for 5-FU. In these experiments, tumor-bearing mice were treated with PM-8 10 times at one-day intervals and with 5-FU 5 times at one-day intervals.

Statistical analysis: The significance of the results was determined with Student's t-test. P values greater than 0.05 were considered insignificant.

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 polyoxonolybdates of PM-8, 17, 26 and 32. The activities of the polyoxomolybdates were compared with those of the refer-

Table 1. Antitumor activities of polyoxometalates against CO-4 human cancer xenografts under the renal 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	〃	1.07±0.01	0.69±0.09 (54.3)[c]
PM-17	25	〃	0.70±0.05	0.87±0.06 (42.4)[c]
PM-26	50	〃	1.02±0.02	1.11±0.05 (26.5)[c]
PM-32	100	〃	1.03±0.01	1.06±0.04 (29.7)[b]
5-F	30	days 1 & 4 (i.p.)	0.99±0.01	0.85±0.02 (44.0)[a]
MMC	2	〃	1.04±0.01	0.83±0.02 (44.9)[a]
ACNU	10	〃	1.01±0.01	0.94±0.09 (37.6)[b]
ADM	5	〃	0.97±0.02	0.87±0.02 (42.4)[a]
CDDP	2	day 1-day 5 (i.p.)	0.97±0.03	0.82±0.03 (46.6)[a]

[1]At day 6 after tumor implantion, tumor sizes of mice treated chemicals were compared to those of vehicle control mice.

[2]PM-8: $[NH_3Pr^i]_6[Mo_7O_{24}] \cdot 3H_2O$, PM-17: $[NH_3Pr^i]_6[H_xMo_7O_{24}]$ $(x=1-2)$, PM-26: $[NH_3Pr^i]_6[Mo_7O_{26}] \cdot 3H_2O$, PM-32: $Na_5[IMo_6O_{24}] \cdot 34H_2O$.

[3]Mean ± SE of five mice. [4]Parenthesis indicate % inhibition of tumor growth.

a), b), and c) indicate significant differences between the values for relative tumor sizes of vehicle control mice versus treated mice ($P<0.001$, $P<0.01$ and $P<0.02$, respectively).

ence antitumor drugs in Table 1 where doses of 1/5 MTD of each compound were used in the screening. Both PM-8 and PM-17 showed significant activities. 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 in SRC assay

The activities of PM-8 against MX-1 and OAT were compared with those of 5-FU (Fig.1). The doses of PM-8 were 200 mg/kg (1/5 MTD) and 100 mg/kg (1/10 MTD), and the dose of 5-FU was 30 mg/kg. PM-8 exhibited significant antitumor activities against both human cancer xenografts. Against MX-1, the activities of PM-8 were equal to those 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.

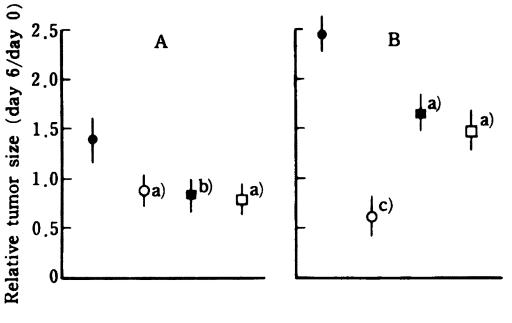

Fig. 1. Antitumor effect of PM-8 against human cancer xenografts implanted under the renal capsule in mice. A. MX-1 (breast cancer), B. OAT (lung cancer). At day 6 relative tumor sizes of saline control mice (closed circle) were compared to those of mice treated i.p. 30 mg/kg of 5-FU at days 1 and 4 (open circle), of mice treated i.p. 100 mg/kg of PM-8 from day 1 to 5 (closed square) or of mice treated i.p. 200 mg/kg of PM-8 from day 1 to 5 (open square). Mice were in groups of five. Bars indicate SE. a), b) and c) indicate significant differences between saline control group and treated groups ($P<0.02$, $P<0.01$ and $P<0.001$, respectively).

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

The antitumor activities of PM-8 when given in multiple doses to nude mice were investigated. Nude mice bearing human cancers were treated i.p. with PM-8 ten times at one-day intervals. 5-FU was given i.p. 5 times at one-day intervals. The antitumor activities of PM-8 are compared with those of 5-FU (Fig. 2).

PM-8 showed significant antitumor activities against MX-1 or OAT human cancer in nude mice. PM-8 was more active against MX-1 than against OAT. The PM-8 activities were equal those of 5-FU in MX-1 bearing mice, but the antitumor activities of PM-8 against OAT were lower than those of 5-FU. However, the treatment with 5-FU caused significant body weight loss in the host mice. PM-8 did not induce any adverse effects such as body weight loss in the host 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 tumor xenografts using SRC assay in mice. Especially, PM-8 was the most active. The geometry of $[Mo_7O_{24}]^{6-}$ for PM-8, consisting of seven MoO_6 octahedra condensed by edge-sharing into the structure is shown in Fig. 3. The character of PM-8 is a water soluble and much stable in water. It is notable that PM-8 showed significant antitumor activities against human tumor 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. Par-

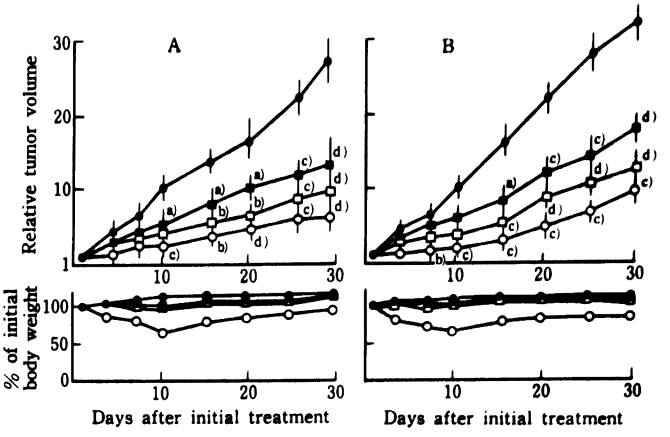

Fig. 2. Antitumor effect of PM-8 against human cancer xenografts in nude mice. A. MX-1 (breast cancer), B. OAT (lung cancer). Mice were treated i.p. 10 ml/kg of saline 10 times at one-day intervals (closed circle), 30 mg/kg of 5-FU 5 times at one-day intervals (open circle), 100 mg/kg of PM-8 10 times at one-day intervals (closed square) and 200 mg/kg of PM-8 10 times at one-day intervals (open square), Mice were in groups of five. Bars indicate SE. a), b), c) and d) indicate significant differences between saline control group and treated groups ($P<0.05$, $P<0.02$, $P<0.01$ and $P<0.001$, respectively).

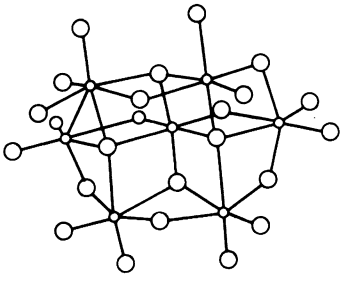

Fig. 3. Structure of $[Mo_7O_{24}]^{6-}$. Large open circles represent oxygen atoms and small open circles represent Mo atoms.

ticularly, 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[16,17].

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. Since PM-8 exhibited excellent antitumor activities in human tumor-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: (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 $[H_xMo_7O_{24}]^{6-}$. Antitumor activities of these moedified compounds against Meth A sarcoma were inventigated.

$[NH_4]^{6-}[Mo_7O_{24}]\cdot 4\,H_2O$ and $K_6[Mo_7O_{24}]\cdot 4\,H_2O$ were effective, whereas $[NH_3Pr^i]Cl$ was ineffective against Meth A tumor bearing mice. Especially, $[NH_3Pr^i]^{6-}[H_xMo_7O_{24}]$ exhibited a significant inhibition on the growth of Meth A sarcoma[9]. The

results indicate that the polyoxomolybdate structure of the Mo_7O_{24} is apparently of critical significance for the antitumor activity.

The toxicities (LD_{50}) of them are 1,850 mg/kg and 250 mg/kg for PM-8 and PM-17 administered intraperitoneally in mice. Furthermore, when they administered i.p. 1/10 LD_{50} once daily for five consecutive days in mice, PM-17 caused severe body weight loss, suggesting that 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 intraction with DNA[18]. Thus PM-8 can be distinguished from the antitumor mechanism of a mononuclear metal complex such as CDDP and other organometalic compounds, since CDDP the dissociation of chloride ligands is followed by binding to N-7 atoms of guanine bases in DNA and formation of DNA interstrand crosslinks at clinically achievable concentration[19]. PM-8 stimulated delayed type hypersensitivity response in mice bearing murine tumor and suppressed the tumor cell proliferation in culture. Probably, PM-8 may act as harmoney with a direct inhibitory activity of cell proliferation and a host mediated effect (unpublished observation). Further studies to explain the detailes of the antitumor mechanism of polyoxomoribdates are in progress.

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新しい型の抗腫瘍物質，ポリオキソモリブデート

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ポリオキソメタレートは金属酸化物の無機重合体である。これら化合物の医学領域への応用を意図し，抗腫瘍活性を検討した。その中でポリオキソモリブデートに抗腫瘍活性が認められ，特に $[NH_3Pr^i]_6[Mo_7O_{24}] \cdot 3H_2O$ (PM-8) が強い抗腫瘍活性を示した。CO-4（ヒト大腸癌）のcd-1マウスにおける腎皮膜下移植系（SRC assay）に対しては5-FU，MMC，ACNU，ADMおよびCDDPと同程度か，やや強い抗腫瘍効果が得られた。さらにMX-1（ヒト乳癌）とOAT（ヒト肺癌）のcd-1マウスにおけるSRC assayとBalb/c，nu/nuマウスにおける移植系に対しては5-FUとほぼ同程度の抗腫瘍効果が得られた。ポリオキソモリブデートは新しい型の抗腫瘍物質である。

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