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ANTITUMOR ACTIVITY OF VINYL POLYMER IMMOBILIZED 5-FLUOROURACILS THROUGH ORGANOSILICON-AMINE SPACER GROUPS VIA UREA BONDS†

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†Dedicated to Otto Vogl on the occasion of his 65th birthday.

ABSTRACT

The homopolymerization of vinyl monomer immobilized 5-fluorouracils (5FU) through organosilicon-amine spacer groups via urea bonds (KY-20) and the copolymerization of KY-20 with vinyl monomer were carried out by a radical technique to give the homopolymer [Poly(KY-20)] and copolymer [Poly(KY-20/vinyl monomer)], respectively. The antitumor activities of Poly(KY-20) and Poly(KY-20/vinyl monomer) were tested against p388 *lymphocytic leukemia* by intraperitoneal (*i.p.*) transplantation/*i.p.* or oral (*p.o.*) administration and against Meth-A *fibrosarcoma* or MM46 *mammary adenocarcinoma* in mice by subcutaneous (*s.c.*) implantation/*p.o.* administration. These 5FU/organosilicon-amine hybrid polymer conjugates exhibited significant survival effects against p388 *leukemia* in mice *i.p./i.p.* or *i.p./p.o.* Poly(KY-20/HPMA) also showed stronger growth-inhibitory effects against Meth-A *fibrosarcoma* and MM46 *mammary adenocarcinoma* in mice *s.c./p.o.*

INTRODUCTION

5-Fluorouracil (5FU), an antimetabolite, is one of the most prominent clinical antitumor agents [1–3]. However, its undesirable side-effects have also been cited [4, 5]. Some kinds of 1-carbamoyl derivatives of 5FU were found to be effective for treatment of mice with L-1210 *leukemia* by oral (*p.o.*) administration [6, 7].

On the other hand, Toyoshima et al. reported that some low molecular weight aminosilanes, such as 3-[(3-trimethylsilyl)propyl] thiopropylamine, administered orally to BDF_1 mice, inhibited the growth of rodent solid tumors, B-16 *melanoma, sarcoma*-180, and *Ehrlich carcinoma* but were ineffective against L1210 and p388 *lymphocytic leukemia* cells [8–10]. Although the action of these aminosilanes is assumed to be due to the dual effect of inhibiting cell growth or activating a delayed-type cellular immunity, its mechanism has not yet been clearly elucidated.

By considering the results described above, we previously synthesized the hybrid vinyl polymers immobilized 5FUs through organosilicon-amine spacer groups via urea bonds, Poly(KY-20) and Poly(KY-20/vinyl monomer), and evaluated their antitumor activities *in vivo* [11, 12]. Consequently, Poly(KY-20) and the copolymer of KY-20 with 2-hydroxyethylmethacrylate, Poly(KY-20/HEMA), were found to exhibit especially higher survival effects against p388 *leukemia* in mice *i.p./i.p.* than free 5FU.

The present paper concerns the antitumor activity of poly(KY-20/2-hydroxypropylmethacrylate (HPMA)), the effect of the molecular weight of Poly(KY-20), and the hybrid effect of organosilicon-amine and 5FU groups on antitumor activity against p388 *leukemia* in mice *i.p./i.p.* This paper also deals with the antitumor activities of Poly(KY-20), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) against p388 *leukemia*, Meth-A *fibrosarcoma*, and MM46 *mammary adenocarcinoma* in mice by oral (*p.o.*) administration.

EXPERIMENTAL

Materials

2-Hydroxyethylmethacrylate (HEMA) and 2-hydroxypropylmethacrylate (HPMA) were purified by distillation in a stream of nitrogen before use. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from methanol. Benzene, pyridine, and tetrahydrofuran (THF) were purified by the usual methods. The other materials were of commercial grade and used without further purification.

KY-20 monomer was prepared by the same method reported previously [11, 12].

Synthesis of Poly(KY-20) and Poly(KY-20/Vinyl Monomer)

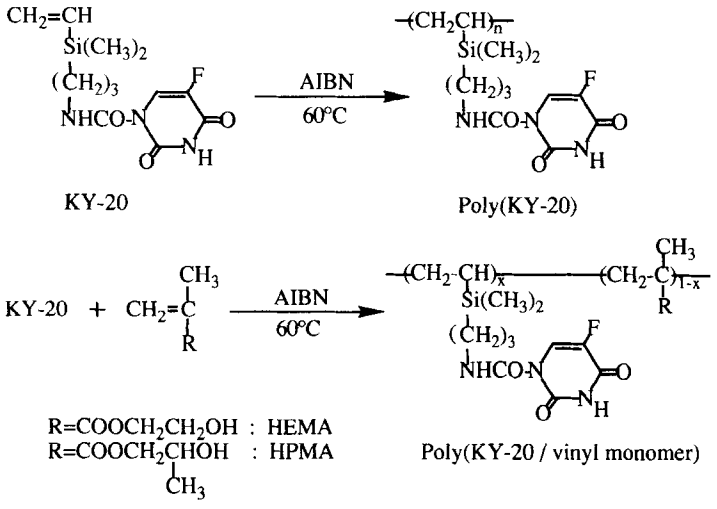
Poly(KY-20) and Poly(KY-20/vinyl monomer) were synthesized by the homopolymerization of KY-20 and the copolymerization of KY-20 with the corresponding monomers, respectively (Scheme 1). The required amounts of KY-20, AIBN, and solvent (THF or benzene) were charged into a glass ampule, which was then degassed by conventional freezing and thawing techniques, and sealed off under vacuum. The polymerizations were carried out at 60°C on standing and then proceeded homogeneously and heterogeneously in THF and benzene, respectively. The precipitated homopolymer of KY-20 [Poly(KY-20)] was thoroughly washed with methanol and reprecipitated from a THF–methanol system.

A similar procedure was applied to the radical copolymerizations KY-20 with HEMA and HPMA in benzene or THF. Diethyl ether was used as a precipitating agent for the copolymers of KY-20 with HEMA and HPMA.

Synthesis of Poly(KY-59/HEMA) and Poly(KY-20(-5FU)/HEMA)

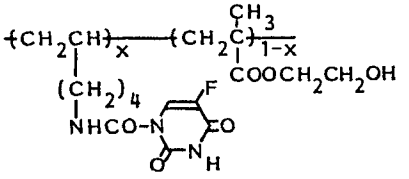
Poly(KY-59/HEMA) was synthesized by the method reported previously [12]; D5FU = 27 mol%.

Poly(KY-20(-5FU)/HEMA) was prepared through hydrolysis of Poly(KY-20/

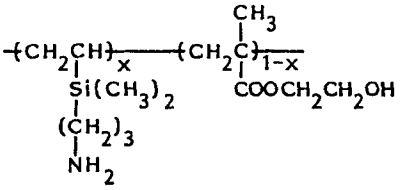


SCHEME 1.

HEMA). A small amount of 0.01 *N* NaOH aqueous solution was added to Poly(KY-20/HEMA) dissolved in THF and then the mixture was stirred at room temperature for 6 h. After concentration, the reaction mixture was poured in a large amount of diethyl ether to give Poly(KY-20(-5FU)/HEMA). By measurements of UV, VH-NMR spectra, it was confirmed that Poly(KY-20(-5FU)/HEMA) could be obtained.



Poly(KY-59/HEMA)



Poly(KY-20(-5FU)/HEMA)

Characterization of Polymers

Poly(KY-20) and Poly(KY-20/HPMA) were water insoluble, and Poly(KY-20/HEMA) was swollen in water.

Since all urea bonds in Poly(KY-20/HEMA) and Poly(KY-20/HPMA) could be perfectly hydrolyzed by refluxing in 5 *N* NaOH aqueous solution for 2 days to give the free 5FU, the degree of introduction of 5FU in mol% 5FU based on the number of vinyl groups (D5FU) for Poly(KY-20/vinyl monomer) was determined by means of UV differential spectrum measurement of the amount of 5FU released in hydrolyzed solution (standard: 6500 of ϵ_{max} for anionic 5FU in 5 *N* NaOH aqueous solution) according to our previously reported method [11]. The number-average molecular weight (M_n) and the weight-average molecular weight (M_w) of Poly(KY-20), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) were measured in THF by GPC methods [column: Gel GPC KF-802 + TSK Gel (G3000H8 + G4000H8 + G5000HXL), detector: UV_{265}, standard: polystyrene].

Measurement of the Antitumor Activities

The antitumor activities were evaluated by four methods.

The survival effect was tested against p388 *lymphocytic leukemia* in female CDF_1 mice (30 untreated mice/group and 6 treated mice/group) *in vivo i.p./i.p.* according to a typical protocol of the Japanese Foundation for Cancer Research (JFCR). 1×10^6 *leukemia* cells were injected *i.p.* on Day 0. The samples were sonicated in 0.05% sorbate 80 in a sterile normal saline solution and administered

i.p. The mice received doses of 200–800 mg/kg twice for the conjugate at 1 and 5 days. The survival effects of the test mice, ILS (%), for the conjugates were evaluated by using the values of median survival of treated mice (T) and that of the control (C) from Eq. (1):

$$\text{ILS}\,(\%) = T/C\,(\%) - 100 \tag{1}$$

These survival effect data are the results of the screening performed at the Cancer Chemotherapy Center of JFCR.

The survival effect was also tested against p388 *lymphocytic leukemia* in CDF_1 mice *in vivo i.p./p.o.* according to the method of Kyowa Hakka Co. The values of ILS for the conjugates were calculated by Eq. (1).

The growth-inhibitory effect was tested against Meth-A *fibrosarcoma in vitro*. Meth-A cells were grown in Dulbecco-modified MEM supplemented with 10% fetal bovine serum. Poly(KY-20) or 5FU was suspended in 1% sugar ester (P-1570), and then the diluted cells were counted after 48 h of the conjugate treatment. The growth-inhibitory effect was evaluated by Eq. (2):

$$\text{growth-inhibitory effect}\,(\%) = (C - T)/C \times 100 \tag{2}$$

where T = average number of cells in treated mice
C = average number of cells in controlled mice

Moreover, the growth-inhibitory effect was tested against Meth-A *fibrosarcoma* or MM46 *mammary adenocarcinoma* in Female Balb/c mice or C3H/He mice *in vivo s.c./p.o.* Female Balb/c and C3H/He mice were implanted *s.c.* with 1.0×10^5 and 2.0×10^5 cells/mouse on Day 0, respectively. The mice were orally administered the doses of the conjugate suspended in 0.1% sugar ester (P-1570) on Days 1, 5, and 9 (three times). The tumor size was measured after 10 or 21 days. The growth-inhibitory effect by the conjugates against such solid tumor cells was evaluated by Eq. (3):

$$\text{growth-inhibitory effect}\,(\%) = (C - T)/C \times 100 \tag{3}$$

where T = average size of cells in treated mice
C = average size of cells in controlled mice

RESULTS AND DISCUSSION

Synthesis of Objective Polymer

We synthesized Poly(KY-20) by means of radical polymerization of KY-20 in benzene at 60°C using AIBN as an initiator. The results of the homopolymerization of KY-20 are shown in Table 1. The homopolymerization proceeded homogeneously in THF and heterogeneously in benzene. The polymerization reactivity of this monomer and the molecular weight of the homopolymer obtained were very low owing to steric hindrance by the pendant bulky group. The Poly(KY-20) obtained was soluble in many organic solvents but not in water.

The copolymerization of KY-20 with HEMA or HPMA in THF at 60°C for 48 h was carried out. The results of homogeneous copolymerization are summarized in Table 2. The contents of KY-20 unit (D5FU) and the molecular weight in Poly(KY-20/HEMA) and Poly(KY-20/HPMA) obtained were not high.

TABLE 1. Homopolymerization of KY-20[a]

KY-20, mol/L	AIBN, mol/L	Solvent	Time, h	Conversion, %	M_w[b]	M_n[b]
1.6×10^{-1}	2.9×10^{-2}	THF	30	49	1.03×10^{3}	6.75×10^{2}
1.6×10^{-1}	1.5×10^{-2}	THF	40	28	2.00×10^{3}	1.24×10^{3}
1.7×10^{-1}	1.6×10^{-2}	Benzene	48	32	3.00×10^{4}	1.70×10^{4}

[a]Polymerized at 60°C.
[b]Determined by GPC method.

TABLE 2. Copolymerization of KY-20 with HEMA or HPMA in THF[a]

				Copolymer		
KY-20, mol/L	Comonomer, mol/L	AIBN, mol/L	Conversion, %	Mole fraction of KY-20, %[b]	M_w[c]	M_n[c]
6.1×10^{-1}	HEMA, 6.2×10^{-2}	2.9×10^{-2}	33	26	1.84×10^3	1.15×10^3
3.3×10^{-1}	HPMA, 1.7×10^{-1}	1.6×10^{-2}	34	38	4.00×10^3	2.40×10^3

[a]Polymerized at 60°C for 48 h.
[b]Namely D5FU (%).
[c]Determined by GPC method.

Survival Effect for Poly(KY-20), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) against p388 *lymphocytic leukemia* in Mice *i.p./i.p.*

The results of the survival effect for Poly(KY-20) and Poly(KY-20/vinyl monomer) against p388 *leukemia* in mice *i.p./i.p.* are shown in Figs. 1 and 2. Poly(KY-20), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) exhibited higher survival effects than free 5FU against p388 *leukemia* mice *i.p./i.p.*

Moreover, these conjugates did not show a rapid decrease of the weight of the treated mice in the dose ranges shown in Figs. 1 and 2; they did not display an acute toxicity in the high dose ranges. Figure 2 shows the effect of the molecular weight of Poly(KY-20) on the survival effect of p388 *leukemia* mice *i.p./i.p.* The activity decreased with an increase in molecular weight of Poly(KY-20). While the antitumor activity of KY-20 monomer was previously reported to be lower than that of KY-20 polymer [11, 12], the oligomer of KY-20 was found to give a good result. Moreover, in order to investigate the effect of 5FU and silicon-amine units on antitumor activity, the survival effect for Poly(KY-20/HEMA) was compared with the effects for Poly(KY-20/HEMA) and Poly(KY-20(-5FU)/HEMA) against p388 *lymphocytic leukemia* in mice *i.p./i.p.* Although Poly(KY-59/HEMA) and Poly(KY-20(-5FU)/HEMA) exhibited relative survival effects, their activities were not higher than the activity of Poly(KY-20) (Fig. 3). These results suggest that the coexistence of 5FU and silicon-amine units was effective for the appearance of high activity.

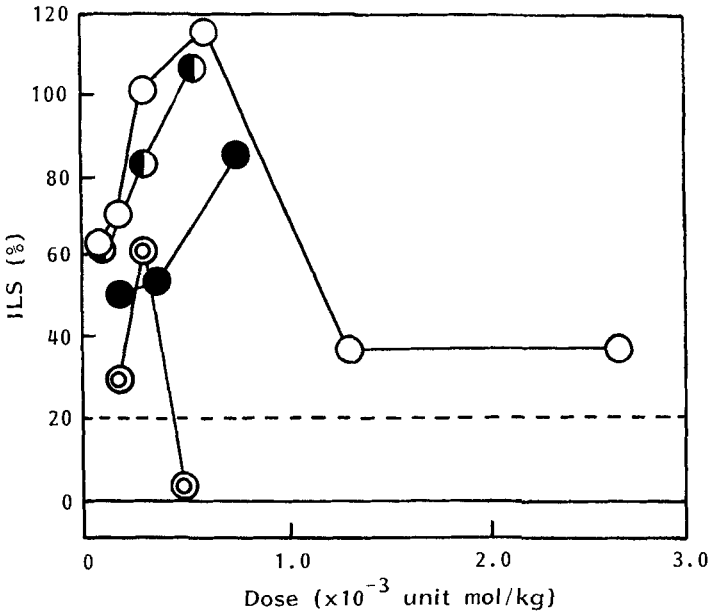


FIG. 1. The prolongation of life for Poly(KY-20) and Poly(KY-20/vinyl monomer) against p388 *lymphocytic leukemia* in mice *i.p./i.p.* (○) Poly(KY-20) (M_w = 1.03 × 10^3); (◐) Poly(KY-20/HEMA) (D5FU = 26 mol%, M_w = 1.84 × 10^3); (●) Poly(KY-20/HPMA) (D5FU = 38 mol%, M_w = 4.00 × 10^3); (◎) 5FU.

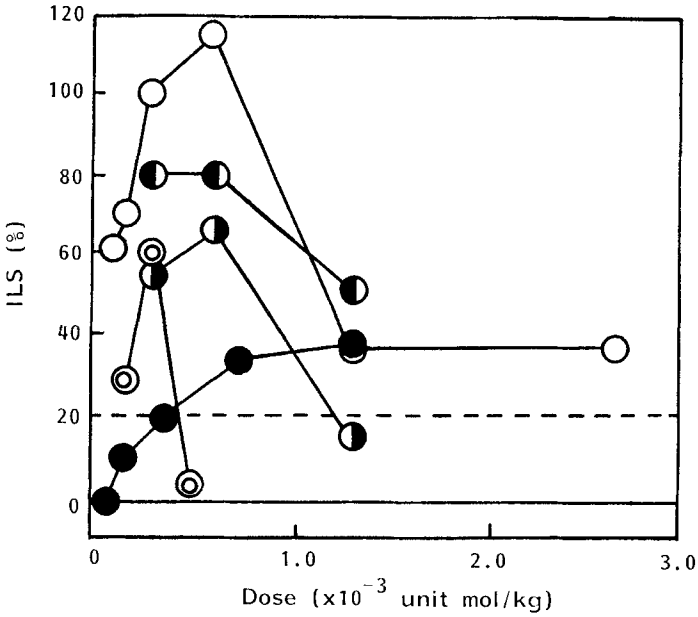


FIG. 2. Effect of the molecular weight of Poly(KY-20) on the prolongation of life against p388 *lymphocytic leukemia* in mice *i.p./i.p.* (○) Poly(KY-20) (M_w = 1.03 × 10^3); (◐) Poly(KY-20) (M_w = 2.00 × 10^3); (◑) Poly(KY-20) (M_w = 3.00 × 10^4); (●) KY-20; (◎) 5FU.

Survival Effect for Poly(KY-20) and Poly(KY-20/HEMA) against p388 *lymphocytic leukemia* in Mice *i.p./p.o.*

Poly(KY-20) and Poly(KY-20/HEMA) against p388 *leukemia* in mice *i.p./p.o.*, compared with that for HCFU and 5FU are shown in Fig. 4. These Poly(KY-20) and Poly(KY-20/HEMA) exhibited higher survival effects against p388 *leukemia* mice *i.p./p.o.* than monomeric HCFU and free 5FU.

Growth-Inhibitory Effect by Poly(KY-20) against Meth-A *fibrocarcinoma* Cells *in vitro*

The results of the growth-inhibitory effect for Poly(KY-20) against Meth-A *in vitro* are shown in Fig. 5. Poly(KY-20) was found to exhibit a significant growth-inhibitory effect against solid tumor cells *in vitro*.

Growth-Inhibitory Effect by Poly(KY-20) and Poly(KY-20/HPMA) against Solid Tumor Cells in Mice *s.c./p.o.*

The results of the growth-inhibitory effect by Poly(KY-20) and Poly(KY-20/HPMA) against Meth-A *fibrosarcoma* or MM46 *mammary adenocarcinoma* in mice *s.c./p.o.* are shown in Figs. 6 and 7. Poly(KY-20) and Poly(KY-20/HPMA) exhibited the same level growth-inhibitory effects against such solid tumor mice by oral

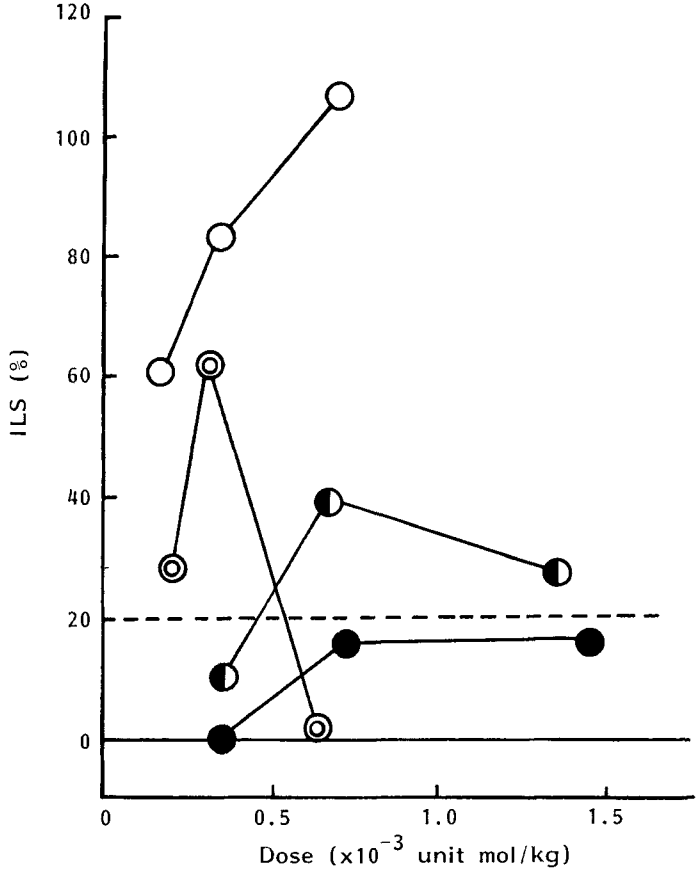


FIG. 3. Effect of 5FU and silicon-amine units on the prolongation of life for vinyl polymer conjugate against p388 *lymphocytic leukemia* in mice *i.p./i.p.* (○) Poly(KY-20/HEMA) (D5FU = 26 mol%, M_w = 1.84 × 10^3); (◐) Poly(KY-59/HEMA) (D5FU = 27 mol%, M_w = 1.96 × 10^3): (●) Poly(KY-20(-5FU)/HEMA) (Dsilicon-amine = 26 mol%, M_w = 1.51 × 10^3); (⊙) 5FU.

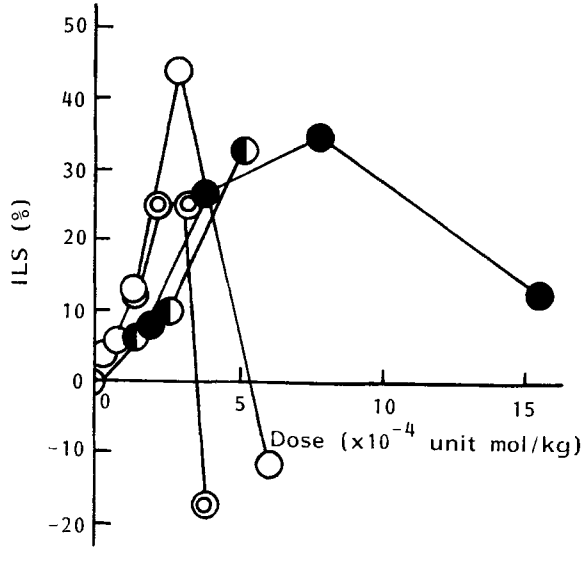


FIG. 4. The prolongation of life for Poly(KY-20) and Poly(KY-20/HEMA) against p388 *lymphocytic leukemia* in mice *i.p./p.o.*, compared with that for HCFU and 5FU. (○) Poly(KY-20) (M_w = 1.03 × 10^3); (◐) Poly-(KY-20/HEMA) (D5FU = 26 mol%, M_w = 1.84 × 10^3); (●) HCFU; (⊙) 5FU.

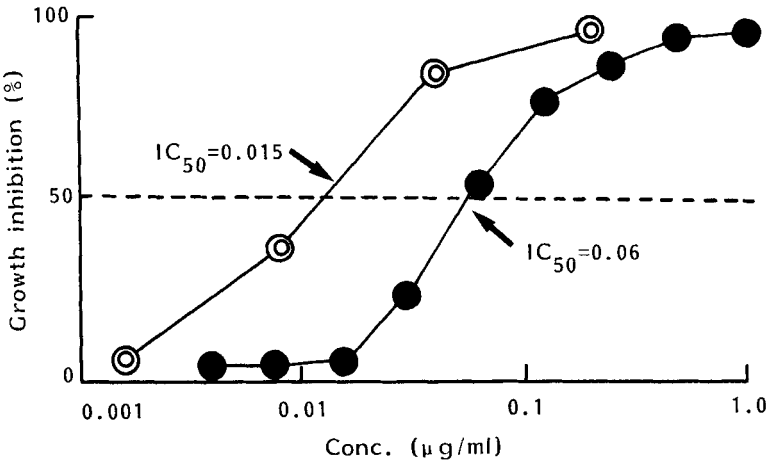


FIG. 5. Growth-inhibitory effect by Poly(KY-20) against Meth-A *fibrocarcinoma* cells *in vitro*. (●) Poly(KY-20) (M_w = 1.03 × 10^3); (◎) 5FU.

administration as those by FT-207, HCFU, UFT, and CDDP used clinically at the present time. These vinyl polymers did not have an acute toxicity to mice even in the high dose ranges.

These results suggest that such a type of bio-undegradable vinyl polymer may be clinically utilized by oral administration against rodent solid tumor.

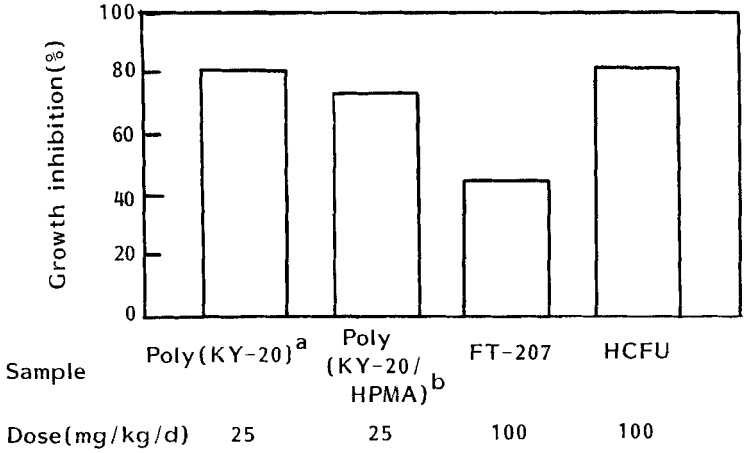
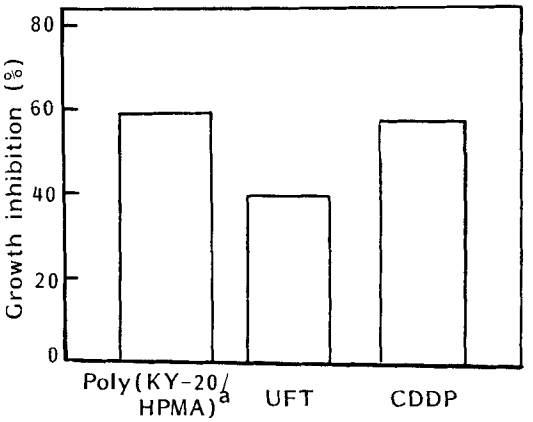


FIG. 6. Growth-inhibitory effect by Poly(KY-20) (M_w = 1.03 × 10^3; D5FU = 38 mol%) and Poly(KY-20/HPMA) (M_w = 4.00 × 10^3) against Meth-A *fibrosarcoma* in mice *s.c./p.o.*, compared with that by FT-207 (Ftoraful) and HCFU (1-hexylcarbamoyl-5FU). After Balb/c mice were implanted *s.c.* with 1.0 × 10^5 cells/mouse, the number of tumor cells was measured after 10 days.



	Poly(KY-20/HPMA)	UFT	CDDP
Dose (mg/kg)	50(p.o.)x3	20(p.o.)x9	1(i.p.)x9
Treatment schedules	D1,5 & 9	D1-D9	D1-D9

FIG. 7. Growth-inhibitory effect by Poly(KY-20/HPMA) (D5FU = 38 mol%, M_w = 4.00 × 10^3) against MM46 *mammary adenocarcinoma* in C3H/He mice *s.c./p.o.*, compared with that by UFT (mixture of uracil and FT-207 (4:1)) and CDDP (*cis*-platin). After C3H/He mice were implanted *s.c.* with 2.0 × 10^5 cells/mouse, the tumor size was measured after 21 days.

CONCLUSION

1. Some kinds of vinyl polymer fixing 5FUs through organosilicon-amine groups via carbamoyl bonds such as Poly(KY-20), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) could be synthesized by means of radical polymerization of the corresponding monomers.

2. Poly(KY-20) (M_w = 1030), Poly(KY-20/HEMA), and Poly(KY-20/HPMA) showed a higher level survival effect than free 5FU against p388 *lymphocytic leukemia* in mice *i.p./i.p.*

3. Poly(KY-20) and Poly(KY-20/HPMA) exhibited higher level growth-inhibitory effects against Meth-A *fibrosarcoma* and MM46 *mammary adenocarcinoma* in mice *s.c./p.o.*

4. The polymer conjugates obtained did not display an acute toxicity in the high dose range *i.p./i.p.* and *s.c./p.o.*

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REFERENCES

[1] S. Waxman and K. J. Scanlon, in E. M. Greenspan (ed.), *Clinical Interpretation & Practice of Cancer Chemotherapy*, Raven Press, New York, 1982, p. 39.
[2] C. Heidelberger, *Cancer Medicine*, 2nd ed., Lea and Febiger, Philadelphia, 1982, p. 801.
[3] C. E. Myers, *Pharmacol. Rev., 1*, 33 (1981).
[4] L. Bosch, E. Harbers, and C. Heidelberger, *Cancer Rev., 18*, 335 (1958).
[5] G. Bounous, R. Pageau, and D. Regoli, *Int. J. Chem. Pharmacol. Biopharm., 16*, 519 (1978).
[6] A. Hoshi, M. Iigo, M. Yoshida, and K. Kuretani, *Gann, 66*, 673 (1975).
[7] A. Hoshi, M. Iigo, A. Nakamura, and K. Kuretani, *Ibid., 67*, 725 (1976).
[8] S. Toyoshima, K. Fukushima, Y. Seto, T. Sakurai, Y. Sugimoto, Y. Yagi, N. Shinohara, Y. Yamamoto, and K. Ito, *Jpn. J. Cancer Chemother., 8*, 579 (1981).
[9] S. Toyoshima, K. Fukushima, T. Sakurai, Y. Sugimoto, H. Sato, and H. Taguchi, *Gann to Kagaku Ryoho, 9*, 225 (1982).
[10] T. Sakurai, K. Fukushima, H. Fujita, and S. Toyoshima, *13th Proc. Int. Congr. Chemother., 16*, 284/135 (1983).
[11] T. Ouchi, K. Hagita, M. Kawashima, T. Inoi, and T. Tashiro, *J. Control. Rel., 8*, 141 (1988).
[12] T. Ouchi, K. Hagita, M. Kawashima, T. Inoi, and T. Tashiro, *J. Bioact. Comp. Polym., 3*, 53 (1988).

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