Macromolecular Research, Vol. 11, No. 5, pp 317-321 (2003)

Preparation and Antitumor Activities of Poly(polyethylene glycol methacrylate-*co*-methacryloyloxymethyl-5-fluorouracil) Prodrug

Suk Hyung Cho*

Division of High-Tech Material and Eectronics, Hyejeon College, Hongsung-eup 350-800, Korea

Kong Soo Kim

Divison of Chemical Engineering, Chungbuk National University, CheongJu 360-763, Korea

Received Mar. 26, 2003; Revised Aug. 4, 2003

Abstract: In order to prepare a prodrug, poly(polyethylene glycol methacrylate-*co*-methacryloyloxymethyl-5-fluorouracil) (poly(PEGM-*co*-MAOFU)) prodrug particles were prepared by precipitation polymerization of MAOFU and PEGM in polyacrylic acid solution. The size of prodrug particles were 0.2-0.35 μm. The antitumor activity of prodrugs against sarcoma-180 tumor cell in mice was demonstrated and the polymer particles themselves showed low toxicity and good biocompatibility when they were administrated into mice.

Keywords: antitumor activity, poly(PEGM-*co*-MAOFU), prodrug, 5-fluorouracil.

Introduction

5-Fluorouracil(5-FU), an antitumor drug, has been widely used for a long time as a chemotherapeutant for adenocarcinoma of stomach, breast, pancreas, head, neck, etc.[1,2] This substance restraints or kills increase of cells because it inhibits DNA synthesis or damages RNA metabolism or function by being inserted into RNA after becoming active. On the other hand, when administered for a long time, it could show side effects such as bone marrow restraint and stomach. Recently, in order to reduce such toxicity, a great deal of researches are being conducted on increasing prescription of drugs by prodrug that is polymerized by being immersed in polymer or increasing the efficacy of a drug by prodrug itself being endocytosis.[3]

For the method, synthesizing 5-FU with a derivative that has 1-carbamoyl, 1-acyloxyalkyl, 1-alkylthio, 1-alkoxyalkyl, etc[4-7] and combining it with natural polymer such as protein, or synthesizing monomer that has drugs of acryloyl type or methacryloyl type and polymerizing it showed high efficacy when administered.[8-10] However, because most of these prodrugs do not dissolve in water due to their high hydrophobicity, they not only are difficult to administer within the body but also cause precipitation or coagulation.[11,12]

Therefore, water soluble prodrug copolymerized with water soluble monomer is produced and administered. However, in this case, it is hard to have enough amount of drugs.[13,14] In order to solve such problem, Yokoyama *et al.*[15] conducted research on prodrug of a new form containing enough amounts of drug as well as stability in the blood by producing PEG/poly (aspartic acid) block copolymer through separation of the part controlling appropriate physical and chemical characteristics and the part of medicinal bond of prodrug, combining it with an antitumor drug, adriamycin (ADR), and forming this into mono-disperse polymer micelle.

In this study, poly(polyethylene glycol methacrylate-*co*-methacryloyloxymethyl-5-FU)(poly(PEGM-*co*-MAOFU)) prodrug particles were prepared by precipitation polymerization of MAOFU and PEGM in polyacrylic acid solution. poly(PEGM-*co*-MAOFU) prodrug has enough amount of drugs as well as good stability in the blood and biocompatibility. Also, the particle sizes and morphology of fine particled prodrug were observed through electron microscopes (SEM) and *in vivo* experiments were conducted to examine antitumor activity.

Experimental

Reagents and Experimental Animals. Methacrylic acid (MAAc) was used after vacuum distillation at 40 °C and polyacrylic acid(M_w: 450,000), PEGM(M_w: 780) and 5-FU were used as purchased. Solvents ethanol and acetonitrile were used after distillation. Methacryloyloxymethyl-5-FU (MAOFU) was synthesized according to a previous method.[4]

For experimental animals used for antitumor experiment,

*e-mail : csh111@hyejeon.ac.kr

1598-5032/10/317-05

6 weeks old male SPF-ICR mice at the weight of 30-40 g were used.

Preparation of Poly(PEGM-*co*-MAOFU) Prodrug. After PEGM and MAOFU was dissolved in polyacrylic acid gel solution, poly(PEGM-*co*-MAOFU) prodrug was prepared by precipitation polymerization in polymer solutin according to a previous method.[16] After polyacrylic acid of fixed quantity with different concentrations was sufficiently swollen by ethanol at room temperature, PEGMA, MAOFU and benzoyl peroxide (BPO) were combined at the ratio of weight to total weight based on the content and left it at 4 °C for 12 hrs before reacting at 60 °C for 24 hrs. To retrieve prodrug particle produced, polyacrylic acid was centrifuged after dissolved by addition of excessive ethanol. White powdered poly (PEGM-*co*-MAOFU) prodrug was obtained after the centrifuged supernatant liquid was subtracted, washed 5 times with ethanol, and dried at room temperature.

Electron Microscope Observation. After spreading powdered polymer prodrug thinly on the grid and vacuum metalizing with gold, its particle sizes and distribution were observed through scanning electron microscope (SEM: Hitachi S-530).

Measurement of Polymer Prodrug Stability. In order to measure stability of polymer prodrug, prodrug was dispersed by 0.1 wt% in 0.2 M-KH_2PO_4 buffer solution and changes of transmittancy was measured at 500 nm through UV/Vis spectrophotometry.

***In vivo* Experiment.** For experimental cell, sarcoma-180 tumor cell which has been transplanted and preserved in the abdominal cavity of ICR mice at the interval of one week at the school of experimental animals of College of Veterinary Medicine of Chungbuk National University was used. That is, on the 8th day of transplantation, the mice filled with ascites were butchered under ether anesthesia and sarcoma-180 tumor cell and ascites were aseptically collected. After 0.83% NH_4Cl-Tris buffer solution was added and mixed carefully, hemolysis of erythrocytes was conducted. Then, after centrifuging at 1,200 rpm for 7 min, remove deserting supernatant liquid, and dispersing pellet carefully, RPMI 1640 medium was centrifuged two times, washed well, and dispersed carefully at appropriate concentrations. The viability of thus separated tumor cells were identified by trypan blue exclusion and the total cell numbers were calculated by hemocytometer.

For evaluation of antitumor activity of sarcoma-180 tumor-bearing mice, 10 mice were included in each group and sarcoma-180 tumor cells were administered in the abdominal cavity of mice at the concentration of 1×10^6/0.2 mL per mouse in all the groups. The samples were administered in the abdominal cavity three times at 4-day intervals at the concentration of 200 mg/kg after 48 hrs from administration of tumor cells. The median survival time (MST) and weight increase according to the progress of sarcoma-180 tumor-bearing mice were observed. In addition, antitumor activity was evaluated from *T*/*C*(%), the ratio of MST(*T*) of experimental group to MST(*C*) of control group.

Results and Discussion

Synthesis and Characteristics of Poly(PEGM-*co*-MAOFU) Prodrug. MAOFU was synthesized according to a previous method.[4] Also, poly(PEGM-*co*-MAOFU) prodrug particles were prepared by precipitation copolymerization of PEGM and MAOFU in polyacrylic acid solution according to a previous new method.[16] MAOFU alone, MAOFU with PEGM in polyacrylic acid/ethanol solution using BPO as initiator were synthesized at 60 °C for 24 hrs and the results are shown in Table I.

As shown in Table I and Figure 1 of SEM, the shapes of synthesized poly(PEGM-*co*-MAOFU) prodrug were identified as particle shapes and the distribution of particles show polydispersibility in very small ranging from 0.2 to 0.35 μm. Prodrug particle size was controlled by reaction conditions.

The result of polymerization with the concentration of polyacrylic acid solution changed to 15-25 wt% is, as shown in Figure 1, that as concentration of polyacrylic acid increases, particle size of microsphere decreases. Such tendency is believed to be because as concentration of polyacrylic acid solution increases, steric inhibition of polymer chain becomes increased restricting the space for poly(PEGM-*co*-MAOFU) to polymerize.

As shown in Figure 2, when the weight percent of MAOFU

Table I. Preparation of Poly(PEGM-*co*-MAOFU) Produg Particles in Polyacrylic Acid/Ethanol Solution

Run NO.	PAA (%)	PEGM (g)	MAOFU (g)	Solvents	Particle Size (nm)	Yields (%)
A-0	20	-	3	ethanol	300	87.5
A-1	25				210	87.0
A-2	20	1	2	ethanol	220	84.0
A-3	15				250	91.0
B-1			1		200	86.5
B-2	20	1	3	ethanol	280	84.0
B-3			4		350	81.0

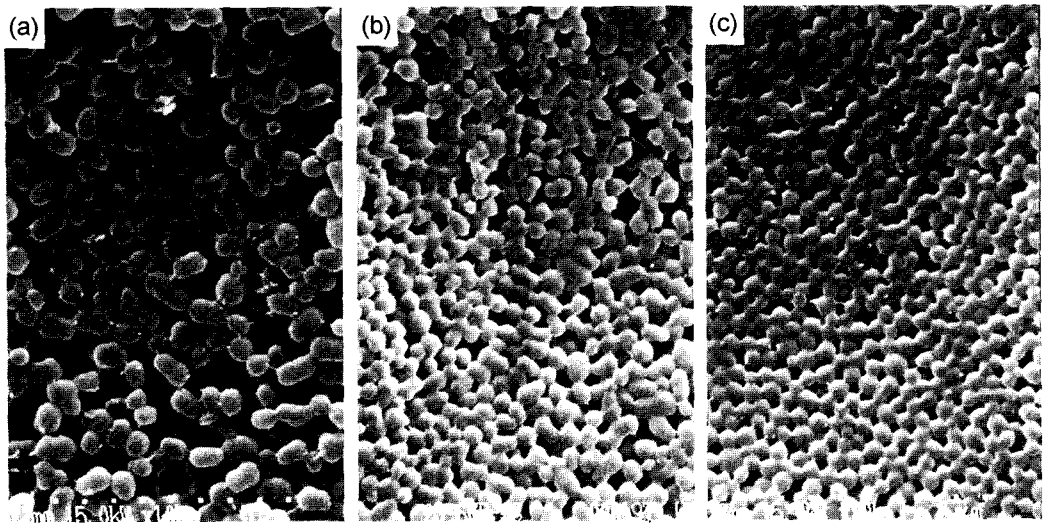

Figure 1. Scanning electron micrographs of poly(PEGM-*co*-MAOFU) prodrug according to poly(acrylic acid) concentration (A: 15 wt%, B: 20 wt%, C: 25 wt%).

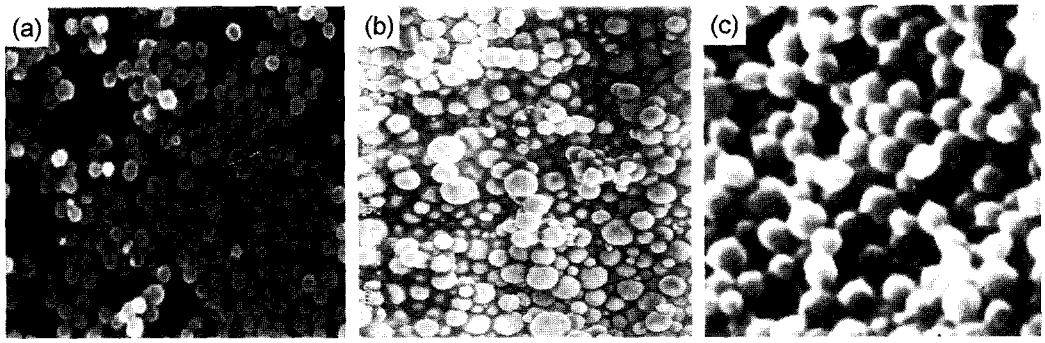

Figure 2. Scanning electron micrographs of poly(PEGM-*co*-MAOFU) prodrug prepared with different monomer concentration (A: 5 wt%, B: 10 wt%, C: 20 wt%).

were changed to 520% respectively, the particle size of microsphere tended to increase within the range of 0.2-0.25 μm as the weight percent of monomers increased. This is believed to be because as concentration of monomer increases, microsphere size increases by monomers doping into the nucleus produced during polymerization.

Stability of Fine Particle Prodrug: Polymer prodrug should restrain absorption of protein to the utmost until reaching target cell or target organ and should not produce flocculation precipitation in the blood. Therefore, stability of fine particle prodrug of polymer is very important in pH like blood.

This research examined stability of fine particle prodrug at pH 7.4 and the result (Figure 3) showed high stability in the buffer solution of pH 7.4. This is believed to be because PEGM of shell part of fine particle functioned as a stabilizer.

Thus, the fine particle prodrug synthesized in this experiment is believed to be a new system that can contain enough amount of drug as well as preserve stability in the blood.

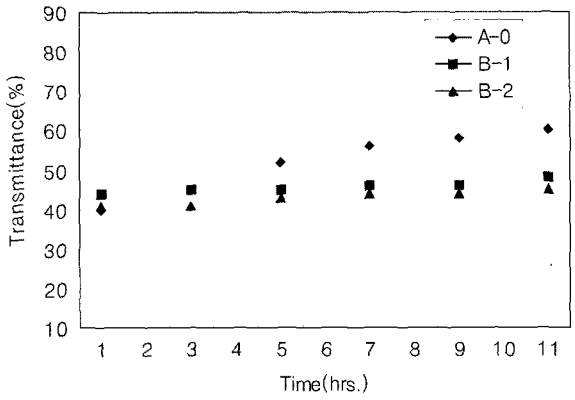

Figure 3. Stabilities of poly(PEGM-*co*-MAOFU) prodrugs in buffer solution of pH 7.4.

***In vivo* Antitumor Activity.** To examine characteristics

of drug release in *in vivo*, sarcoma-180 tumor cells were transplanted in the abdominal cavity of 6-week-old male ICR mice and the samples of 5-FU, A-0, B-1, B-2, and B-3 were administered three times at 4-day intervals at the concentration of 200 mg/kg after 48 hrs from administration of tumor cells. Since prodrug used in *in vivo*, antitumor activity is expected to have no effect or general effect. However, two circumstances can be considered in this experiment. The one is that the activity might not appear because absence of enzyme decomposition causes failure of reduction to the original drug (parents) by release from prodrug, and the other is that drug release can be inhibited, due to existence of protein or phosphatide within the body.[17]

Table II and III show changes of weight in sarcoma-180 tumor-bearing mice and effect of antitumor activity. In case 5-FU administration group showed 47% increase compared to the control group. B-1, B-2 and B-3 in which hydrophilicity was copolymerized showed 47, 60, 62% of prolongation of life compared to the control group.

Moreover, 5-FU administration group showed relatively high antitumor activity but as revealed in Table II, it showed decrease of weight. Such decrease of weight is believed to be due to the side effect by 5-FU toxicity. On the other hand, in case prodrug was administered, it is believed toxicity did not appear judging from that weight increased.

Looking into the effect of A-0 on sarcoma-180 tumor-bearing mice, PEGM was not copolymerized at polymer prodrug. From the facts that A-0 showed almost same survival time as the control group but the rate of survival time in B-1, B-2, B-3 was 47% above than that of control group, it is believed that when PEGM is combined, low toxicity and good biocompatibility show. The difference for *T/C* is due to difference of amount of drug released from prodrug particle relatively. The rate of penetration of polymer into cell depends on the mechanism of pinocytosis. This mechanism may also be affected by the polymer structure. Synthetic polymer particles having hydrophilic surface are taken up cells having endocytotic activity such as tumor cells.[18,19] This fact that the high antitumor activity obtained in poly(PEGM-*co*-MAOFU) resulted from combination of 5-FU and PEGM.

Table II. Changes of Body Weight in Mice Injected with Various Drugs

Treatment	Body Weight (g)			Weight Change (g/mouse)
	7days	10days	15days	
control	33.5±2.4	42.1±4.2	55.3±5.6	+21.8
5-FU	34.3±2.9	32.2±2.4	32.1±2.4	-2.2
A-0	35.2±2.4	43.5±4.4	54.3±5.4	+19.1
B-1	34.5±3.4	42.5±3.7	52.4±5.6	+17.9
B-3	36.2±3.4	46.5±5.7	54.4±5.6	+18.2

Table III. Effect of Various Drugs Against Sarcoma-180 Tumor-Bearing mice

Treatment	MST (day)	*T/C* (%)	Long Survivors[a]
control	5.85	100	0
5-FU	8.60	148	0
A-0	6.15	106	0
B-1	8.52	147	1
B-2	9.31	160	1
B-3	9.50	162	2

[a]45-day survivors are included as long survivors.

Conclusions

In this research, poly(PEGM-*co*-MAOFU) prodrug particle was synthesized by precipitation polymerization and its characteristics and ability as a drug deliverer were examined. The followings are the conclusion of the results.

1. Fine particle polymer prodrug of 0.2-0.35 μm was obtained in polyacrylic acid/ethanol solution. The prodrug was not monodisperse but polydisperse and poly(PEGM-*co*-MAOFU) prodrug particle have good water dispersibility by the influence of PEGM.

2. As the result of antitumor activity of *in vivo* experiment using sarcoma-180 tumor bearing mice, the prodrug showed relatively high effect of antitumor activity than 5-FU alone and did not have toxicity by side effect.

References

(1) S. Ahmad, S. Ozaki, T. Nagase, M. Ligo, and A. Hoshi, *Chem. Pharm. Bull.*, **35**, 4137 (1987).
(2) M. Hasida, A. Sato, T. Kojima, S. Muranishi, H. Sezaki, N. Tanigawa, K. Satomura, and Y. Hikasan, *Jpn. J. Cancer Res.*, **72**, 226 (1981).
(3) H. Onishi, T. Gawaguchi, and T. Nagai, *Chem. Pharm. Bull.*, **35**, 3370 (1987).
(4) T. Ouchi, H. Yuyama, and Oogl, *J. Marcromol. Sci. Chem.*, **A24**, 1011 (1987).
(5) T. Yang and R. Zhno, *Polym. J.*, **22**, 572 (1990).
(6) M. Akashi, N. Miyauchi, N. Morita, and T. Minota, *J. Bioact. Compat. Polym.*, **2**, 232 (1987).
(7) S. Ozaki, Y. Watanabe, T. Hoshico, T. Nagase, T. Ogasawara, H. Furukawa, A. Uemura, K. Ishikawa, M. Mori, M. Iigo, and R. Tokuzen, *Chem. Pharm. Bull.*, **34**, 150 (1986).
(8) S. H. Cho, K. S. Kim, J. K. Kang, and B. H. Cho., *Polymer (Korea)*, **18**, 877 (1994).
(9) F. Yong and R. Zhuo, *Polym. J.*, **22**, 572 (1990).
(10) Y. Tusukuda, K. Okawa, and N. Hibi, *Cancer Res.*, **47**, 4293 (1987).
(11) S. Ozaki, J. Ohnishi, Y. Watanabe, T. Nohda, T. Nagase, T. Akiyama. N. Uehara, and A. Hoshi, *Polym. J.*, **19**, 955 (1987).
(12) Y. Kita, Y. Imaki, and K. Takemoto, *J. Polym. Sci., Polym.*

Chem. Ed., **18**, 427 (1981).
(13) N. Endo, N. Umemoro, Y. Koto, and T. Hara, *J. Immunol. Methods*, **101**, 253 (1987).
(14) S. Ozaki, J. Ohnishi, T. Akiyama, T. Nagase, N. Uehara, and A. Hoshi, *Polym. J.*, **22**, 689 (1990).
(15) M. Yokoyama, M. Miyauchi, N. Yamada, T. Okano, Y. Sakurai, and K. Kataoka, *Cancer Res.*, **50**, 1695 (1990).
(16) S. H. Cho and K. S. Kim, *Macromol. Res.*, in press (2003).
(17) T. Gawaguchi, A. Tsugane, and K. Higashide, *J. Pharm. Sci.* **81**, 508 (1992).
(18) A. J. Sbarra, W. Shirley, and W. A. Bardowil, *Nature*, **194**, 255 (1962).
(19) R. Duncan and J. Kopecek, *Adv. Polym. Sci.*, **57**, 51 (1984).