[Chem. Pharm. Bull. 30(10)3770—3775(1982)]

Antitumor Effect of Ethylene-Vinyl Acetate Copolymer Matrices containing 5-Fluorouracil on Ehrlich Ascites Carcinoma in Mice[1,2]

Shozo Miyazaki,*,[a] Kuniaki Ishii,[b] Kenji Sugibayashi,[b] Yasunori Morimoto,[b] and Masahiko Takada[a]

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University,[a] Ishikari-Tobetsu, Hokkaido 061-02, Japan and Faculty of Pharmaceutical Sciences, Josai University,[b] Sakado, Saitama 350-02, Japan

(Received May 19, 1982)

Ethylene-vinyl acetate (EVA) copolymer was evaluated as a carrier for controlled release of 5-fluorouracil (5-FU). In order to study the effect of comonomer ratio modifications on the drug release kinetics, the release of 5-FU dispersed in polymer matrices composed of different ratios of ethylene and vinyl acetate was investigated. The vinyl acetate content of EVA copolymer was varied from 8 to 40% w/w. An increase in vinyl acetate comonomer content increased the drug release from the polymer matrix. The release rate could be controlled by modifying the ethylene/vinyl acetate ratios in the polymer matrices.

The antitumor activity of EVA copolymer matrices containing 5-FU was evaluated against Ehrlich ascites carcinoma in mice, on the basis of changes in body weight and animal survival data. Tumor cell injections were performed on Day 0 and matrix implantations on Day 4, both intraperitoneally. The suppressive effect of matrices containing 5-FU on the increase in body weight was higher than that of the free drug. A prolongation of the life-span of tumor-bearing mice following implantation of therapeutic matrices was also noted.

These results indicated that EVA matrices containing 5-FU may be effective in cancer chemotherapy. Matrices composed of EVA copolymer could be useful vehicles for implanted, inserted, or surface-applied delivery systems for anticancer agents.

Keywords——ethylene-vinyl acetate copolymer; drug carrier; drug delivery; controlled release; 5-fluorouracil; Ehrlich ascites carcinoma; mice

Recently, much effort has been made to enhance the antitumor effect of anticancer agents for cancer chemotherapy. One possible approach for increasing the effectiveness of anticancer agents might be the selective administration of a controlled release preparation into cancerous lesions. Unlike conventional routes of drug administration, controlled release systems that use implanted, inserted, or surface-applied polymeric vehicles can deliver a steady quantity of drug to a target area over long periods of time. A variety of polymer membranes or matrices have been employed as rate-controlling barriers in such systems for anticancer agents, including silicone rubber,[3] polyethylene,[4] vinyl polymer,[5] hydroxypropyl cellulose,[6] hydro-gels,[7] polylactic acid,[8] and polyglycolic acid.[9]

Ethylene-vinyl acetate (EVA) copolymer is a heat-processable, flexible, and inexpensive material.[10] The safety and biocompatibility[11] of EVA copolymer are reflected in its use as a biomaterial for artificial hearts[12] and as an antithrombogenic material.[13] In the previous paper,[14] it was demonstrated that the release rate for a drug could be easily controlled by modifying ethylene/vinyl acetate ratios in the copolymer matrix.

The usefulness of EVA copolymer as a drug delivery system for pilocarpine, progesterone, hydrocortisone,[15] fluoride ion,[16] and macromolecules such as proteins[17] was described. However, no reports have dealt with the release of anticancer agents from EVA copolymer matrices.[18] The present investigation was undertaken to determine by means of *in vitro* experiments the amounts of a potent anticancer agent, 5-fluorouracil (5-FU), released from EVA copolymer matrices. The antitumor activity of EVA copolymer matrices containing 5-FU was evaluated against Ehrlich ascites carcinoma in mice.

Experimental

Materials——5-Fluorouracil (5-FU) was obtained from Sigma Chemical Co., St. Louis, and used without further purification. Ethylene-vinyl acetate copolymers (EVAFLEX) with various comonomer ratios were gifts from Mitsui Polychemical Co., Tokyo.

Matrix Preparation——A weighed amount of drug powder was dispersed in 100 ml of methylene chloride or toluene in a glass vial. EVA copolymers (5 g) were dissolved in the drug suspension at 50°C, except that a copolymer with 8% w/w vinyl acetate content was dissolved in the toluene solution. This mixture was poured onto a glass plate and the solvent was allowed to evaporate off at room temperature overnight. The membrane was removed from the plate and dried for 2 d at room temperature *in vacuo*. The residue was placed in a steel mold and melt-pressed at 100°C under 500 kg/cm^2 pressure for 2 min to produce a membrane of uniform thickness. Then, rectangular matrices were cut from the membrane and weighed accurately. The drug content was calculated from the weight ratio of drug and copolymer used.

***In Vitro* Release Studies**——The matrices prepared by the above procedure were placed separately in 20 ml vials containing 10 ml of distilled water. The drug release was followed with shaking at a rate of 60 strokes/min on the incubator at 37°C. Each matrix was successively transferred to fresh vials containing 10 ml of water. The amount of 5-FU released from the EVA copolymer matrix was measured spectrophotometrically at 266 nm. Data shown in the figures are averages of three experimental runs.

Animal Experiments——ICR mice, weighing approximately 30 g, were used in the animal experiments. For evaluating the antitumor effect of the EVA matrix, ICR mice were inoculated intraperitoneally with 2×10^6 Ehrlich ascites carcinoma cells. Four days after inoculation, the mice were implanted with EVA copolymer matrix containing 5-FU or EVA copolymer matrix without drug. The mice were anesthetized with pentobarbital and a small incision was made through the skin on the abdomen of the mice; the matrix was inserted intraperitoneally and pushed away from the incision area. The incisions were sutured and the animals placed in a cage. The mice were injected with 0.3 ml of 5-FU suspension in 0.9% NaCl solution (free 5-FU). Changes in body weight and survival time of treated, tumor-bearing mice were recorded.

Results and Discussion

In Vitro Controlled Release of 5-FU from the EVA Copolymer Matrices

For studying the effect of comonomer ratio changes on the drug release kinetics, the release of 5-FU dispersed in matrices composed of different ratios of ethylene and vinyl acetate

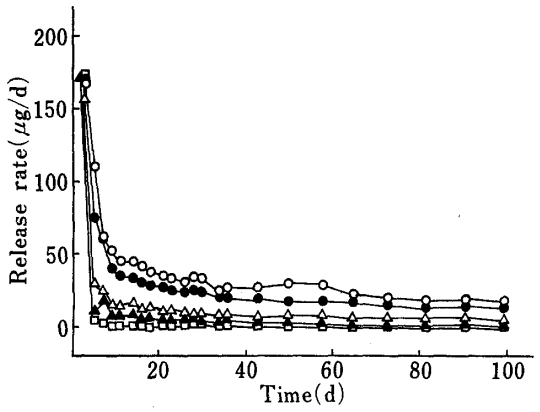

Fig. 1. Release Rate of 5-FU from EVA Copolymer Matrices at 37°C (13.1 mg of 5-FU per Matrix)

Symbols; □, 8; ▲, 19; △, 25; ●, 33; ○, 40% w/w vinyl acetate. The average size of matrices was 1.46× 1.12× 0.23 cm (n=15).

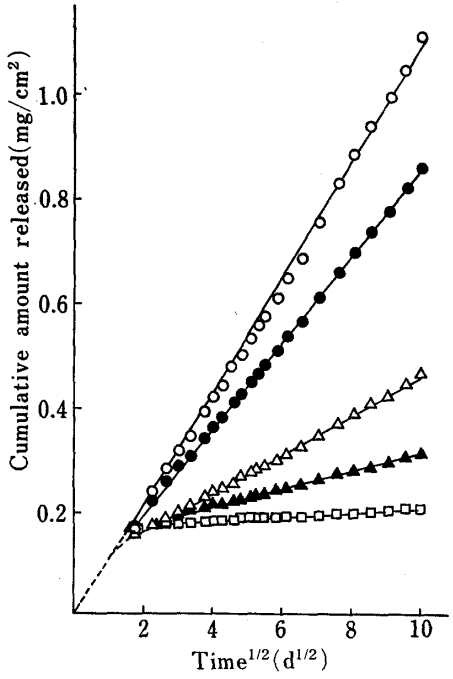

Fig. 2. Cumulative Release of 5-FU from the EVA Copolymer Matrices

Symbols; see Fig. 1.

was investigated. EVA copolymers ranging from 8 to 40% w/w of vinyl acetate unit were used in this study.

The mean daily release rates from matrices are shown in Fig. 1. In all cases, release rates were much higher than the basal level during the first few days of release; this is called the "burst effect." There was a continuing decrease in the amount of drug released daily over the next 30 d; then the release became constant. Thus, 5-FU release was maintained at about 18, 13, 6, 3, and 1 μg/d from the matrices prepared with copolymers of 40, 33, 25, 19, and 8% w/w vinyl acetate content for a period of over 100 d.

Figure 2 shows plots of the data, expressed as the cumulative amount of the drug released (Q) *versus* the square root of time ($t^{1/2}$).[19] After an initial period of rapid release of the drug, the release was approximately linear with respect to $t^{1/2}$. The steady-state rate of drug release (k) was estimated from the slope of the linear Q-$t^{1/2}$ profile and is shown in Fig. 3 as a function of the vinyl acetate content. Increasing the vinyl acetate content from 0 to 20% w/w affected the release rate only slightly. Beyond 20% w/w, there was a marked increase in release rate with further increase in the vinyl acetate content. The *in vitro* release of 5-FU from the EVA matrices continued for longer than the 100-d test period. The total amount of 5-FU released during the period was 24.8% of the dose for the matrix prepared with copolymer containing 40% w/w vinyl acetate.

Thus, it is expected that with this system, the drug release rate could be easily controlled by modifying the proportion of ethylene and vinyl acetate.[18] It should also be pointed out that sustained release can be obtained by using EVA copolymer containing less vinyl acetate.

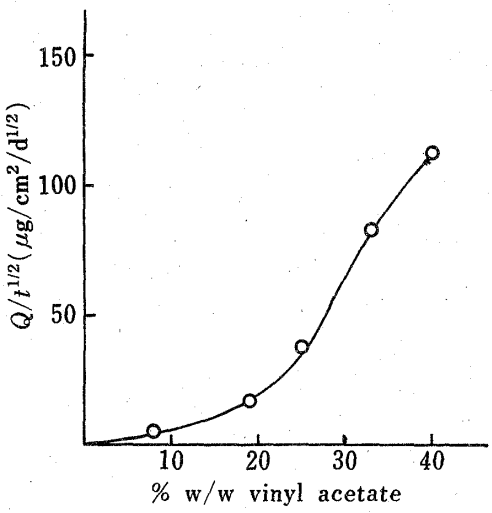

Fig. 3. Release Rate Constants of 5-FU as a Function of Vinyl Acetate Content of EVA Copolymer Matrices

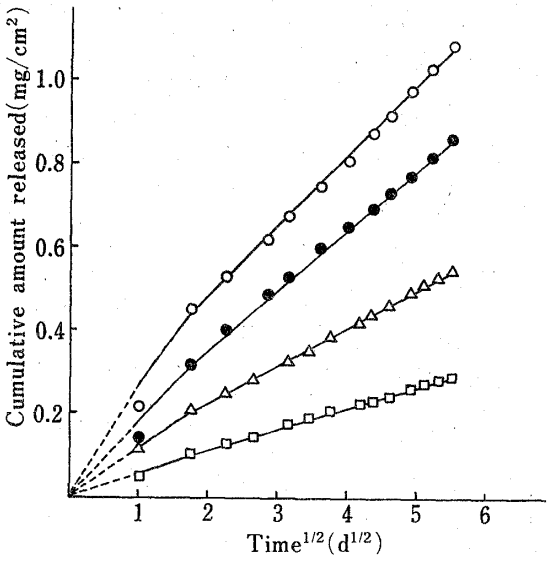

Fig. 4. Effect of Initial Drug Content on the Cumulative Release of 5-FU from the EVA Copolymer Matrix with 40% w/w Vinyl Acetate Content at 37 °C

Symbols; □, 1.9; △, 3.7; ●, 5.1; ○, 9.3 mg per matrix. The average size of matrices was 1.44×1.10×0.03 cm (n=12).

The effect of drug concentration on the release rate was tested using four concentrations of 5-FU (1.9, 3.7, 5.1, and 9.3 mg per matrix) in matrices prepared with copolymers of 40% w/w vinyl acetate content. As shown in Fig. 4, variation in the initial drug content of the matrix affects the drug release; increasing the drug content increases the drug release rate. The k values for the matrices with 1.9, 3.7, 5.1, and 9.3 mg of the drug were 48.4, 87.5, 134.8, and

160.4 $\mu g/cm^2/d^{1/2}$, respectively. The release rates were also shown to be proportional to drug concentration.

A wide spectrum of release rates can be achieved by altering the comonomer ratio and the drug content. Thus, EVA copolymers are potentially useful vehicles for the practical controlled release of anticancer agents.

Antitumor Activity of the EVA Copolymer Matrices containing 5-FU against Ehrlich Ascites Carcinoma

Although 5-FU has been found to be active against Ehrlich ascites carcinoma,[20] it is not very effective due to rapid elimination. The antitumor activity of EVA copolymer matrix containing 5-FU was therefore evaluated against Ehrlich ascites carcinoma in mice. Matrices containing either 3.7 mg or 9.3 mg of 5-FU per matrix were prepared from copolymers of 40% w/w vinyl acetate content. Tumor cell injections were performed on day 0 and matrix implantations on day 4, both intraperitoneally.

First, the antitumor effect of EVA matrices was evaluated by following the changes in body weight. Figures 5 and 6 show the changes in body weight in mice treated with free 5-FU (10 mice), EVA matrix containing 5-FU (10 mice), and EVA matrix without drug (8 mice). They also include the results in normal and tumor-bearing mice (10 mice). At both doses, the increase in body weight after implantation of the matrices was smaller than that in the group receiving EVA matrix without drug or free 5-FU. This result indicates that sustained drug release occurs in the peritoneum and that effective drug concentrations may be maintained by implantation of the EVA matrices. Thus, EVA matrices containing 5-FU did limit the increase in body weight due to the tumor growth.

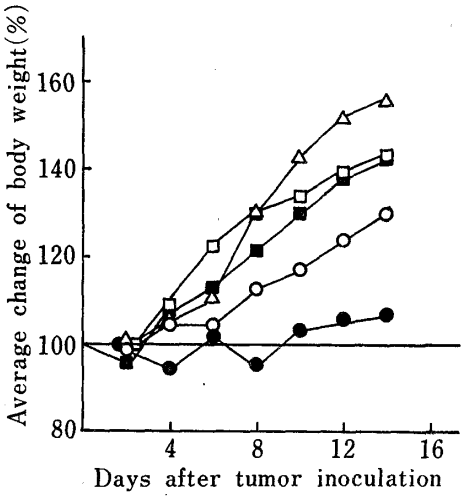

Fig. 5. Changes in Body Weight of Ehrlich Ascites Tumor-bearing Mice (3.7 mg of 5-FU per Matrix)

Symbols; —●—, control (normal mice); —□—, control (tumor mice); —△—, treated with free 5-FU; —■—, treated with EVA matrix without drug; —○—, treated with EVA matrix containing 5-FU.

The average size of matrices was 1.45×1.25×0.03 cm (n=18).

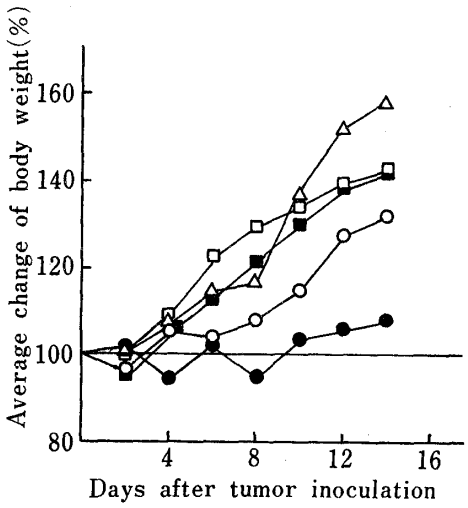

Fig. 6. Changes in Body Weight of Ehrlich Ascites Tumor-bearing Mice (9.3 mg of 5-FU per Matrix)

Symbols; see Fig. 5.

Next, the antitumor effect was evaluated on the basis of animal survival data. Table I summarizes the antitumor effects of free 5-FU, EVA matrices containing 5-FU, and EVA matrices without drug against Ehrlich ascites carcinoma.

Non-treated tumor-bearing mice died between 14 and 21 d after inoculation and the mean survial was 16.2 d. Implantation of EVA matrices without drug did not produce any significant difference in survival from the control. When the mice were treated with 3.7 and 9.3 mg of free 5-FU at day 4 after inoculation, the mean survival times were 17.6 and 18.8 d and T/C was 108 and 116%, respectively. In contrast, when EVA matrices containing 3.7 and 9.3 mg of 5-FU were implanted into the mice, the mean survival increased to 21.4 and 24.9 d and T/C was 132 and 153%, respectively; these values are higher than that obtained with free 5-FU. One out of 10 mice survived over 60 d after implantation of the matrices containing 9.3 mg of 5-FU; the mean survival time of the other 9 rats was 21.0 d and T/C was 129.6%.

TABLE I. Effect of intraperitoneally Administered 5-FU or EVA Copolymer Matrices on Ehrlich Ascites Carcinoma in Mice

Treatment	Dose	Survival (d)[a]	T/C[b]	Number of mice survived/treated
Control	—	16.2±0.8	—	0/10
EVA matrix without drug	—	16.8±0.9	103.7	0/8
Free 5-FU	3.7 mg	17.6±0.9	108.6	0/10
	9.3 mg	18.8±0.8	116.0	0/10
EVA matrix containing 5-FU	3.7 mg	21.4±2.1	132.1	0/10
	9.3 mg	24.9±12.1	153.7	1/10
		(21.0±3.1)[c]	(129.6)[c]	

a) Mean value±S.E.
b) Mean survival of treated mice/mean survival of control.
c) Calculated for 9 mice that survived for less than 60 d.

These results indicate that implantation of EVA matrices containing 5-FU may be effective in cancer chemotherapy. EVA shows good biocompatibility and should be useful as a prolonged-release drug carrier for implanted, inserted, or surface-applied delivery systems for anticancer agents.

Acknowledgement The authors are grateful to Mitsui Polychemical for the supply of ethylene-vinyl acetate copolymers (EVAFLEX).

References and Notes

1) Pharmaceutical Application of Biomedical Polymers, Part VII. Part VI: S. Miyazaki, K. Ishii, and M. Takada, *Yakuzaigaku*, accepted.
2) This work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982.
3) M.L. Rosenblum, D.L. Bowie, and M.D. Walker, *Cancer Res.*, **33**, 906 (1973); M.F. Refojo, H.S. Liu, F.-L. Leong, and D. Sidebottom, *J. Bioeng.*, **2**, 437 (1978).
4) Y. Sato, M. Morita, K. Miyakawa, K. Inoue, E. Akido, and S. Doi, *Jap. J. Cancer Clin.*, **21**, 1159 (1975).
5) I. Kaetsu, M. Yoshida, and A. Yamada, *J. Biomed. Mater. Res.*, **14**, 185 (1980).
6) Y. Machida, H. Masuda, N. Fujiyama, S. Ito, M. Iwata, and T. Nagai, *Chem. Pharm. Bull.*, **27**, 93 (1979).
7) M. Arlen, J.W. Scherrer, J. Cukierman, F. Nagin, and B.S. Levowitz, *Arch. Surg.*, **105**, 100 (1972); J. Drobnik, P. Spacek, and O. Wichterle, *J. Biomed. Mater. Res.*, **8**, 45 (1974); S. Hosaka, Y. Murao, and H. Nakagawa, *Membrane*, **5**, 377 (1980).
8) S. Yolles, T.D. Leafe, and F.J. Meyer, *J. Pharm. Sci.*, **64**, 115 (1975).
9) M. Hirano, R. Wakasa, T. Ichihashi, Y. Watanabe, T. Iwa, M. Nakahara, and K. Sakai, *Artificial Organs*, **11**, 221 (1982).
10) A. Furumiya, *Kagaku Kogyo*, **30**, 1006 (1967); S.P. Halbert, A.E. Ushakoff, and M. Anken, *J. Biomed. Mater. Res.*, **4**, 549 (1970); A. Watanabe, Y. Iwai, and E. Masuhara, *Artificial Organs*, **4**, 14 (1975).
11) R. Langer, H. Brem, and D. Tapper, *J. Biomed. Mater. Res.*, **15**, 267 (1981).
12) P.N. Sawyer, M. Page, B. Rudewald, H. Langergren, L. Baselius, C. McCool, W. Halperin, and S. Srinivasan, *Trans. Am. Soc. Artif. Intern. Organs*, **17**, 470 (1971).

13) H. Miyama, N. Harumiya, Y. Mori, and H. Tanzawa, *J. Biomed. Mater. Res.*, **11**, 251 (1977); T. Oshiro, Y. Kido, Y. Ogawa, G. Kosaki, and K. Takagi, *Artificial Organs*, **10**, 989 (1981).
14) S. Miyazaki, K. Ishii, and T. Nadai, *Chem. Pharm. Bull.*, **29**, 2714 (1981).
15) J.C. Johnson(ed.), "Sustained Release Medications," Noyes Data Co., Park Ridge, 1980, pp. 96—97; S.W. Kim, R.V. Petersen, and J. Feijen, "Drug Design," Vol. X, ed. by E.J. Ariëns, Academic Press, New York, 1980, pp. 217—219.
16) B.D. Halpern, O. Solomon, L. Kopec, E. Korostoff, and J.L. Ackerman, "Controlled Release Polymeric Formulations," ed. by D.R. Paul and F.W. Harris, American Chemical Society, Washington D.C., 1976, pp. 135—146.
17) R. Langer, D.S.T. Hsieth, W. Rhine, and J. Folkman, *J. Membr. Sci.*, **7**, 333 (1980).
18) S. Miyazaki, K. Ishii, and M. Takada, *Yakuzaigaku*, accepted.
19) T. Higuchi, *J. Pharm. Sci.*, **50**, 874 (1961).
20) C. Heidelberger, L. Griesbach, B.J. Montag, D. Mooren, O. Cruz, R.J. Schnitzer, and E. Grunberg, *Cancer Res.*, **18**, 305 (1958).