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Effect of implanted ethylene-vinyl alcohol copolymer matrices containing 5-fluorouracil on Ehrlich ascites carcinoma

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The antitumour activity of ethylene-vinyl alcohol (EVAl) copolymer matrices containing 5-fluorouracil (5-FU) was evaluated against Ehrlich ascites carcinoma in mice. A prolongation of the life-span of tumour-bearing mice following intraperitoneal implantation of therapeutic matrices was noted. These results indicated that EVAl matrices containing 5-FU may be effective in cancer chemotherapy. Matrices composed of EVAl copolymer could be useful vehicles for implanted, inserted, or surface-applied delivery systems for anticancer agents.

To maximize the effectiveness of anticancer agents and to minimize their toxic side effects, topical administration of a controlled release preparation into cancerous lesions has been attempted. 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 synthetic polymer membranes or matrices have been employed as rate-controlling barriers in such systems, including silicone rubber (Rosenblum et al 1973), hydro-gels (Arlen et al 1972), polyethylene (Sato et al 1975), polylactic acid (Yolles et al 1975), vinyl polymers (Kaetsu et al 1980), and ethylene-vinyl acetate (Miyazaki et al 1982). Only limited work has been reported with the use of implantable polymer/drug composites in the treatment of tumour-bearing animals.

Ethylene-vinyl alcohol (EVAl) copolymers prepared from ethylene-vinyl acetate (EVAc) copolymers are non-toxic, flexible, and heat-processable. The unique characteristic of this copolymer, different from EVAc copolymer, is its hydrophilicity (Yamashita et al 1979). The safety and biocompatibility of the copolymer are reflected in its use as a haemodialysis membrane (Hoshino et al 1978). Physicochemical properties of the EVAl copolymer can be varied over a wide range by means of changes in the comonomer ratios (Iwasaki & Hoshino 1977). EVAl can be applied for the controlled release of hydrophilic drugs because of its hydrophilic character. However, no attention appears to have been directed to EVAl copolymer as a drug carrier except for our studies (Miyazaki et al 1981; 1983a, b).

In the previous paper (Miyazaki et al 1983b), it was demonstrated that the release rate for a potent anticancer agents, 5-fluorouracil (5-FU), could be easily controlled by modifying ethylene/vinyl alcohol ratios in the EVAl copolymer matrices. The present investigation was undertaken to evaluate the antitumour activity of implanted EVAl copolymer matrices containing 5-FU against Ehrlich ascites carcinoma in mice.

Materials

5-Fluorouracil (5-FU) was obtained from Sigma Chemical Co., St Louis and used without further purification. Ethylene-vinyl alcohol (EVAl) copolymers with 43, 54 and 60 mol% of ethylene unit were gifts from Kuraray Co., Tokyo.

Methods

Preparation of the EVAl copolymer matrices

Controlled release 5-FU copolymer matrices were prepared based on the method of Miyazaki et al (1983b). They were fabricated in the shape of a disk, 1 cm diameter and 0·2 cm thick. The EVAl copolymer (2 g) and the required amount of drug were dissolved in 40 ml of solvent (n-propanol–water, 3 : 1) at 80–85 °C. This mixture was poured onto a polyester film and the solvent was allowed to evaporate. The residue obtained was melt-pressed in a conical mould to produce a disk of

† Correspondence.

uniform thickness. The drug content was calculated from the weight ratio of drug and copolymer used.

Measurement of release rate

The prepared controlled release matrices were placed in 15 ml vials containing 5 ml of distilled water. The drug release was followed with shaking at a rate of 60 strokes min^{-1} on the incubator at 37 °C. At each time, each matrix was successively transferred with forceps to fresh vials containing 5 ml of water. The released 5-FU concentration was determined spectrophotometrically by measuring the absorption at 266 nm. Release studies were made in triplicate and the average values were plotted.

Animal experiments

Male ddY mice, ~32 g, were used. For evaluating the antitumour effect of the EVAl matrix, ddY mice were inoculated intraperitoneally with 2×10^6 Ehrlich ascites carcinoma cells. Two (3·9 mg 5-FU) or three (7·2 mg 5-FU) days after inoculation of the cells, the mice were implanted with EVAl copolymer matrix containing 5-FU or EVAl copolymer matrix without drug. The mice were anaesthetized with pentobarbitone 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 skin incision was sutured and the animals placed in a cage under constant temperture and humidity. The mice were injected with 0·3 ml of 5-FU suspension in 0·9% NaCl (saline) (free 5-FU). The antitumour effect was evaluated on the basis of animal survival data.

Results and discussion

In-vitro release of 5-FU from the EVAl 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 alcohol was investigated. EVAl copolymers with 43, 54 and 60 mol% of ethylene unit were used.

Fig. 1 shows plots of the data, expressed as the cumulative amount of the drug release (Q) versus the square root of time ($t^{\frac{1}{2}}$). After an initial period of rapid release of the drug, the release was approximately linear with respect to $t^{\frac{1}{2}}$. The steady-state rate of drug release (k) was estimated from the slope of the linear Q-$t^{\frac{1}{2}}$ profile (Higuchi 1961). The k values for the matrices with 43, 54 and 60 mol% ethylene contents were 39·46, 25·22 and 13·25 $\mu g\ cm^{-1}\ h^{-\frac{1}{2}}$, respectively. The effect of increasing ethylene content in the copolymer matrix was to cause a decrease in the release rate for 5-FU. The in-vitro release of 5-FU from the EVAl matrices continued for longer than the 8-day test period. The total amount of 5-FU release during the period was 20·7, 12·8 and 7·2% of the dose for the matrices prepared with copolymer containing 43, 54 and 60 mol% ethylene, respectively.

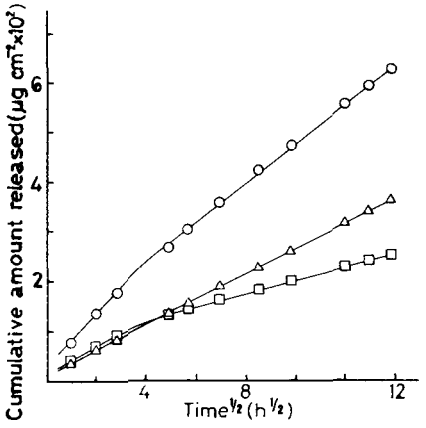

FIG. 1. Cumulative 5-FU release from the EVAl copolymer matrices at 37 °C (7·2mg of 5-FU per matrix): ○, 43; △, 54; □, 60 mol% ethylene.

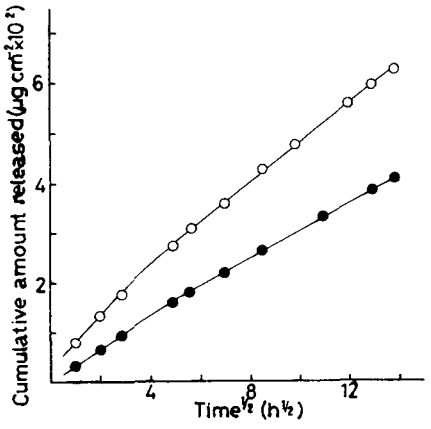

FIG. 2. Cumulative 5-FU release from the EVAl copolymer matrices with 43 mol% ethylene at 37 °C: ●, 3·9; ○, 7·2 mg of 5-FU per matrix.

Table 1. Effect of EVAl copolymer matrices containing 5-FU and free 5-FU on survival time of mice bearing Ehrlich ascites carcinoma. Ehrlich ascites carcinoma (2×10^6 cells) was inoculated intraperitoneally into ddY mice. Chemotherapy was given with intraperitoneal single injection (free 5-FU) or implantation (EVAl matrices).

Compound	Dose (mg per mouse)	Mean survival time (days)	T/C[a] (%)	Survivors at 60 days
Control	—	18·2 (1·3)	100·0	0/6
Free 5-FU	3·9[b]	22·2 (1·6)	122·0	0/6
	7·2[c]	20·5 (0·9)	112·6	0/6
EVAl matrix without 5-FU	—[c]	20·7 (0·7)	113·7	0/6
EVAl matrix containing 5-FU	3·9[b]	43·8 (3·7)*	240·7	1/6
	7·2[c]	41·7 (5·4)**	229·1	0/6

[a] Calculated as the ratio of the mean survival time of the treated group divided by that of the control group.
[b] Chemotherapy was given at 2 day after inoculation.
[c] Chemotherapy was given at 3 day after inoculation.
* Significantly different ($P < 0·001$) from the control.
** Significantly different ($P < 0·005$) from the control.

The effect of 5-FU content on release patterns is shown in Fig. 2. A 43 mol percent EVAl matrix was used in this investigation because this would release 5-FU more rapidly than those with higher ethylene contents (Fig. 1). It is evident that the smaller the 5-FU content, the more slowly was the drug released. The steady-state k values for the matrices with 3·9 mg of the drug was 27·69 $\mu g\ cm^{-1}\ h^{-\frac{1}{2}}$.

Thus, it is expected with this sytem, that the drug release rate could be easily controlled by modifying the proportion of ethylene/vinyl alcohol ratio and drug content. It should also be pointed out that sustained release can be obtained by using EVAl copolymer containing more ethylene.

Antitumour activity of the EVAl copolymer matrices

Although 5-FU has been found to be active against Ehrlich ascites carcinoma (Heidelberger et al 1958), it is not very effective due to rapid elimination. The antitumour activity of EVAl copolymer matrix containing 5-FU was therefore evaluated against Ehrlich ascites carcinoma in mice. Matrices containing either 3·9 mg or 7·2 mg of 5-FU per matrix were prepared from the copolymer of 43 mol% ethylene content. Tumour cell injections were made on day 0 and matrix implantations on day 2 (3·9 mg) or day 3 (7·2 mg), both intraperitoneally.

Table 1 summarizes the antitumour effects of free 5-FU, EVAl matrices containing 5-FU, and EVAl matrices without drug against Ehrlich ascites carcinoma. All activities were calculated as T/C %, the ratio of the mean survival time of the treated group (T) divided by that of the control group (C). The mean survival time of the untreated control group was 18·2 day. Implantation of EVAl matrices without drug did not produce any significant difference in survival from the control. When the mice were treated with 3·9 and 7·2 mg of free 5-FU, the mean survival times were 22·2 and 20·5 days, respectively. In contrast, when EVAl matrices containing 3·9 and 7·2 mg of 5-FU were implanted into the mice, the mean survival increased to 43·8 and 41·7 days and T/C was 240·7 and 229·1%, respectively; these values are higher than that obtained with free 5-FU. One of six mice bearing Ehrlich ascites carcinoma given EVAl matrices containing 3·9 mg 5-FU was still surviving 60 days after inoculation. These results indicate that sustained drug release occurs in the peritoneum and that effective drug concentrations may be maintained by implantation of the EVAl matrices. Thus, EVAl matrices containing 5-FU did bring about prolongation of life span of tumour-bearing mice.

These data suggest that implantation of sustained release EVAl matrices containing 5-FU can be an effective means of cancer chemotherapy, as they provide a more convenient method of drug delivery to target tissues while minimizing drug toxicity and maximizing drug effectiveness. EVAl copolymers show good biocompatibility and should be useful as a controlled release drug carrier for implanted, inserted, or surface-applied delivery systems for anticancer agents.

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