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DRUG-CARRIER PROPERTY OF ALBUMIN MICROSPHERES IN CHEMOTHERAPY. III. EFFECT OF MICROSPHERE-ENTRAPPED 5-FLUOROURACIL ON EHRLICH ASCITES CARCINOMA IN MICE

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To examine the possibility of utilizing albumin microspheres as drug-carriers, an *in vitro* release of 5-fluorouracil (5-FU) from albumin microspheres was examined and the effect of intraperitoneally injected drug-carrying microspheres on Ehrlich ascites carcinoma in mice was studied. *In vitro* release characteristics determined by dialysis experiments showed that 5-FU release continued over one week. We also noted that drug release in the peritoneum of ascites-bearing mice continued over one week and that their life-span increased. Furthermore, the microspheres were phagocytized *in vivo* by the ascites cells. Our results suggest that albumin microspheres containing 5-FU may represent an effective system of drug delivery with prolonged action.

Keywords——albumin microsphere; drug-carrier; 5-fluorouracil; prolonged action; phagocytosis; tumor cell

Inert carriers have been used in chemotherapy to direct drugs to target tissues[1] and they have also been utilized to sustain the release of pharmaceuticals.[2] Miller *et al.*[3] showed that polylactate and polyglyconate copolymers were degraded gradually after intraperitoneal injection into rats, and they suggested that the polymers were useful as drug carriers. Arakawa *et al.*[4] reported sustained drug release from liposome suspensions (phospholipid vesicles) and Gregoriadis *et al.*[5,6] suggested that liposome-entrapped drugs were phagocytized preferentially by tumor tissues. These experiments showed the liposome to be an effective drug carrier in cancer chemotherapy. Albumin microspheres have been utilized as a lung or liver scanning agent.[7,8] Kramer suggested the possibility that albumin microspheres could be utilized as effective, tissue-specific drug carriers,[9] and he and his coworkers reported that human serum albumin microspheres containing 6-mercaptopurine were selectively phagocytized *in vitro* by HeLa and glioblastoma cells.[10]

We recently reported that 5-fluorouracil (5-FU) entrapped in bovine serum albumin (BSA) microspheres was present in high levels in the liver of mice after intravenous injection.[11] But it is obscure that albumin microspheres may represent therapeutic effect with prolonged action in cancer chemotherapy. If albumin microspheres containing antitumor agent show the sustained release and prolonged action, then albumin microspheres may provide a new field in cancer therapy. Then we study the sustained release of BSA microsphere-entrapped 5-FU and the prolonged action of the entrapped drug in Ehrlich ascites carcinoma as a model tumor *in vivo*. This preliminary study was undertaken to determine the extended action of the microspheres, to measure the sustained release of 5-FU from microspheres, and to observe the phagocytosis of microspheres by tumor cell *in vivo*.

MATERIALS AND METHODS

Materials——5-Fluorouracil (5-FU) was obtained from Kyowa Hakko Co., Ltd. and bovine serum albumin (BSA) from Seikagaku Kogyo Co., Ltd. ICR mice were used in all animal experiments. Male and female ICR mice, weighing approximately 30 g were intraperitoneally inoculated at 10-day intervals with 2×10^7 Ehrlich ascites.

Preparation of BSA Microspheres——BSA microspheres containing the antitumor agent 5-FU were prepared by a modification of the method of Scheffel *et al.*[8] The final microspheres contain about 3.3% 5-FU.

In Vitro Drug Release——Drug release from the microspheres was determined by a dynamic dialysis system with a cellulose tubing (36/32, Visking Co.) (Fig. 1). One hundred milligrams BSA microspheres containing 5-FU were suspended in isotonic phosphate buffer (pH 7.4). After 10 min ultrasonication using a 2.6 cm titanium probe at 250 μA in a sonicator (Nihonseiki Seisakusho, Model G50022-4) and centrifugation to remove the drug which adhered to the microspheres, the precipitates were resuspended with 3 ml of isotonic phosphate buffer (pH 7.4) and dialyzed against of 47 ml of isotonic phosphate buffer at 37°. The inner solution was stirred at 50 rpm with a stirrer attached to an electric motor and the outer solution was stirred at 200—300 rpm with an acrobat stirrer (Emuesukiki Co., Ltd.).

Samples (1 ml) were withdrawn from the outer solution at certain intervals and 1 ml of buffer was added to keep the volume constant. Drug adsorption to the Visking dialysis tube was negligible. The drug concentration of the samples was determined at 266 nm by a spectrophotometer (Model 100-20, Hitachi Co., Ltd.) after the addition of acetate buffer (pH 4.7).[12]

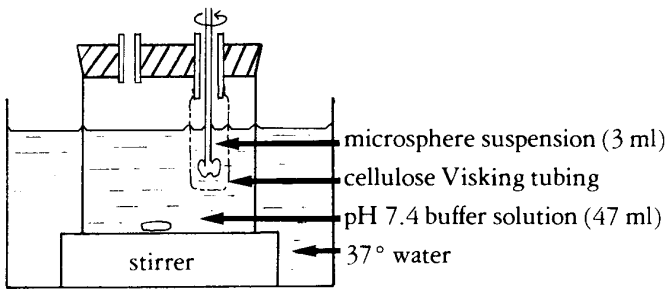


FIG. 1. *Apparatus used to Study the Release of 5-FU from Microspheres*

Toxicity Studies——Toxicity of the microspheres containing 5-FU was determined by animal survival and evidence of rejection in 30 g male mice following intraperitoneal injection. Acute (7 days) and chronic (30 days) evaluations of the toxic effects were performed in 20 mice per group.

Microscopic Study of Phagocytosis——Phagocytic uptake was determined as follows: 24 hr after 2×10^7 ascites inoculation, the mice received an intraperitoneal injection of microsphere (30 mg of microsphere containing 1 mg of 5-FU per mouse) or 0.9% NaCl solution (control) and were killed by cervical dislocation 5 days after inoculation. The ascites cells were collected immediately, fixed in methyl alcohol, stained with Giemsa, and examined under an optical microscope. Alternatively, dehydration, drying and metal-coating of the ascites were carried out, and then the samples were used to take scanning electron micrographs.

In Vivo Drug Release——To determine drug release in the peritoneum and phagocytic uptake by Ehrlich ascites carcinoma, 24 hr after inoculation with Ehrlich ascites cells, the mice were administered with intraperitoneal injection of

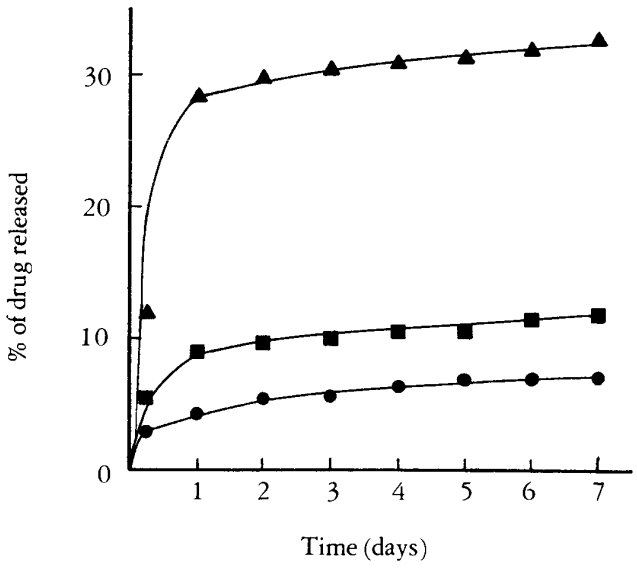


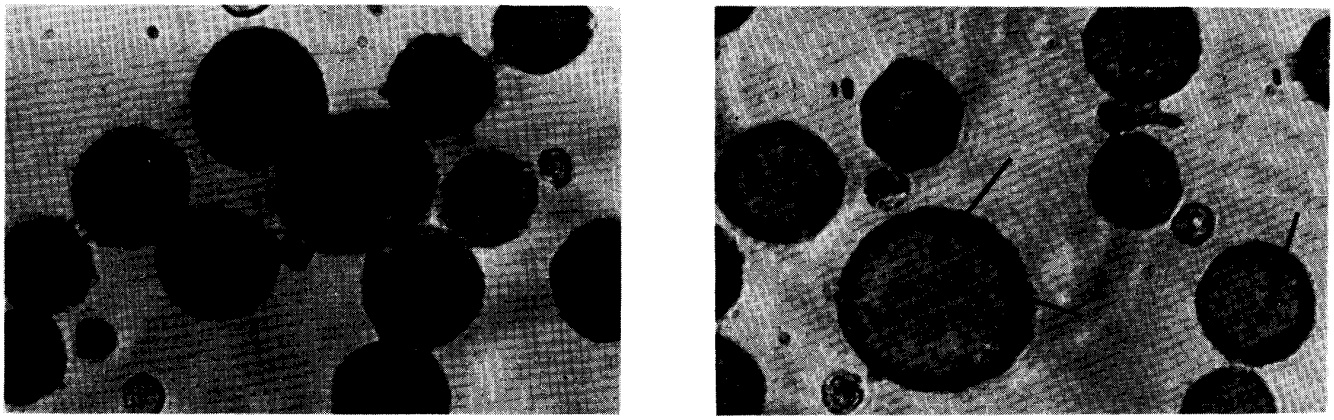
FIG. 2. *Release of 5-FU from Microspheres Albumin microspheres prepared at 180° (●), 150° (■), 100° (▲).*

microspheres containing 5-FU (30 mg of microspheres containing 1 mg of 5-FU per mouse), free 5-FU (1 mg per mouse) or 0.9% NaCl solution (control). Changes in body weight and survival times of treated, tumor-bearing mice were recorded.

RESULTS

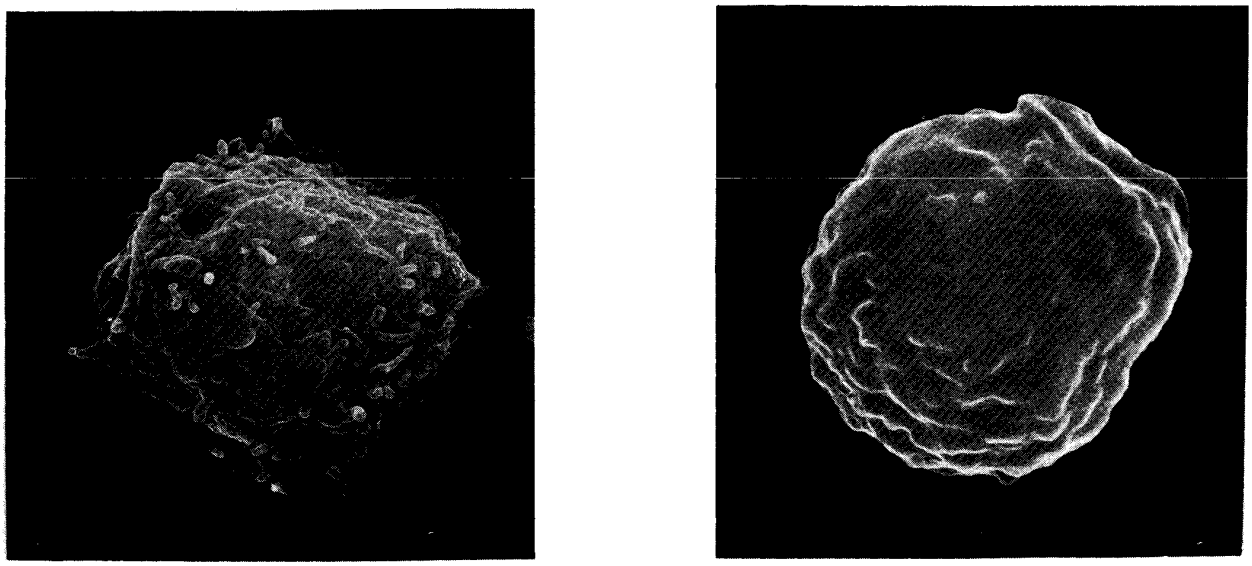
In Vitro Drug Release

As shown in Figure 2, *in vitro* 5-FU release continued over one week, although the release rate was very slow. The level of drug release depended on the temperature of the microsphere preparation, suggesting difference in microsphere structures and hardness levels. Zolle *et al.*[7] reported that swelling occurred in microspheres suspended in a solution, and that the degree of swelling decreased with increase in the temperature of the microsphere preparation. An increase in the temperature of the microsphere preparation increased the hardness of



(a) (b)

FIG. 3. *Micrographs of Ehrlich Ascites Carcinoma*
(a) control, (b) after treatment with albumin microspheres. Arrows indicate microspheres phagocytized by tumor cells. Giemsa stain (magnification ×600)



(a) (b)

FIG. 4. *Scanning Electron Micrographs of Ehrlich Ascites Carcinoma*
(a) control (×6200), (b) after treatment with albumin microspheres containing 5-FU (×14000).

the microspheres, leading to decreased drug release. Consequently, drug diffusion in the microsphere matrix is reduced due to decreased microsphere porosity and increased tortuosity.

Microsphere Toxicity

Negligible side effects were discovered in acute and chronic toxicity studies. None of the 20 mice examined in each type of study died even the highest dosage tested (50 mg microsphere per mouse). No localized ulcerations and/or loss of hair, indicative of rejection, were observed at various dosage levels tested.

Phagocytosis of Microspheres by Ehrlich Ascites Carcinoma

Figure 3 shows micrographs of Ehrlich ascites carcinoma in mice 5 days after injection of albumin microspheres or 0.9% NaCl solution. Figure 3b reveals microsphere phagocytosis by the ascites cells. Kramer and Burnstein,[10] using isotope-labeled drugs entrapped in microspheres, demonstrated the uptake of albumin microspheres by tumor cells *in vitro.* Since the mechanism underlying phagocytosis of macromolecules and microspheres has not been completely elucidated to date, we attempted the additional demonstration of the presence of microspheres in the cell lysate.

Figure 4 shows that intraperitoneal administration of albumin microspheres containing 5-FU effected morphological changes on the surface of Ehrlich ascites cells compared with injection of

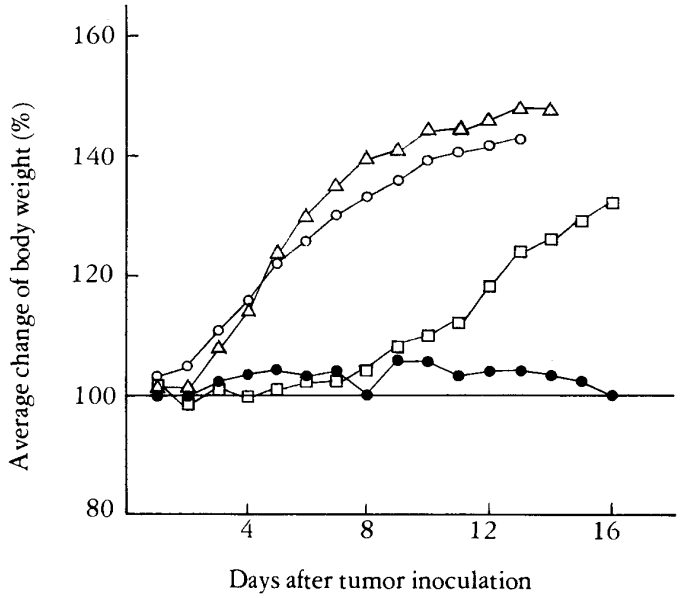


FIG. 5. *Change in Body Weight of Ehrlich Ascites Tumor Bearing Mice*
Treatment with 0.9% NaCl solution (○), free 5-FU (△) and microspheres containing 5-FU (□) normal control bearing no tumor (●)

0.9% NaCl solution. This change may be explained by a sustained abdominal level of 5-FU, by an intracellular concentration of 5-FU due to the endocytic activity of the tumor cells or by both effects.

Effect of Microspheres Containing 5-FU on Ehrlich Ascites

Although 5-FU has been found to be active against Ehrlich ascites carcinoma,[13] its effect was

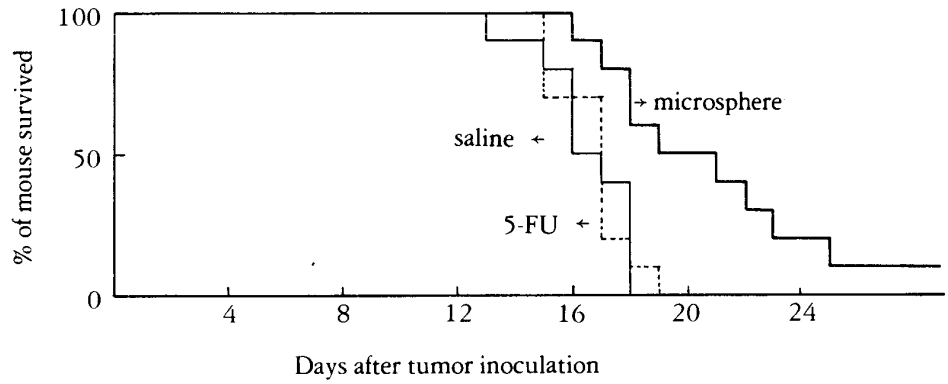


FIG. 6. *Effect of Albumin Microsphere-entrapped 5-FU on the Survival of Mice inoculated with Ehrlich Ascites Carcinoma*
The life span was increased by 1.2% in 5-FU treated mice and 20.5% in mice administered microspheres containing 5-FU.*
**This value represents the average of 9 mice.*

poor due to a fast elimination rate. We further examined the effect of microspheres containing 5-FU in Ehrlich ascites and found that intraperitoneal injection of albumin microspheres containing 5-FU resulted in drug activity against the ascites and the suppression of tumor growth at the inoculation site, lasting for approximately one week. Thereafter, however, tumor growth effected an increase in animal body weight (Figure 5). The increase in life-span effected by microsphere treatment is shown in Figure 6.

DISCUSSION

Zolle *et al.*[7] and Scheffel *et al.*[8] utilized the phagocytic activity of the liver and spleen and radiolabeled albumin microspheres to study and diagnose the function of the reticuloendothelial system. Albumin microspheres were degradated and metabolized and no evidence of antigenicity was found, indicating the effective drug-carrier properties of microspheres. We reported earlier that microspheres injected intravenously into mice were concentrated in the liver due to phagocytosis of the reticuloendothelial system.[11 14]

In the present study we found that *in vitro* release of 5-FU from albumin microspheres continued over one week (Figure 2). Our findings that after microsphere injection, the average body weight of the ascites-bearing mice did not increase for one week, indicate that sustained drug release occurs in the peritoneum and that effective drug concentrations may be maintained for a week (Figure 5). We also noted a prolongation in the life-span of tumor-bearing mice following therapeutic microsphere administration (Figure 6). Our experimental results suggest that albumin microspheres containing 5-FU may represent an effective system of drug delivery with prolonged action.

However, suppression of tumor growth disappeared by one week. This may be due to the fact that the amount of 5-FU delivered in single-shot administration of microspheres was therapeutically insufficient.

Studies are presently under way in our labolatory to examine the effects of multiple dose administration of microspheres containing 5-FU. In addition, we plan to investigate the applicability of the microsphere drug delivery system to several experimental tumor systems.

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