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Antitumor Effect of Fibrinogen Microparticles Containing Adriamycin on Ehrlich Ascites Carcinoma in Mice[1)]

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The antitumor activity of fibrinogen microparticles containing adriamycin against Ehrlich ascites carcinoma in mice was evaluated on the basis of animal survival data. Tumor cell injections were performed on day 0 and microparticle injections on day 1, both intraperitoneally. A prolongation of the life span of tumor-bearing mice following injection of therapeutic microparticles was noted, and the microparticles containing adriamycin were therapeutically more effective than adriamycin alone. The high chemotherapeutic efficiency of fibrinogen–adriamycin microparticles was striking at high doses which would be toxic in the case of the drug alone. These results indicated that fibrinogen microparticles containing adriamycin may be effective in cancer chemotherapy. Microparticles composed of fibrinogen could be a novel drug carrier for use in injectable delivery systems for anticancer agents.

Keywords——fibrinogen microparticle; novel drug carrier; drug delivery system; adriamycin; sustained release; Ehrlich ascites carcinoma; mouse

The possible use of fibrinogen microspheres as a novel drug carrier for injectable delivery systems for anticancer agents has been examined.[3)] In the previous work, during the preparation of fibrinogen microspheres containing adriamycin, large amounts of the polymer tended to give aggregated microparticles rather than microspheres when stabilization of the fibrinogen matrix of the microspheres was accomplished by heat denaturation at 90 °C. On the other hand, fibrinogen microspheres were obtained at higher temperature (140 °C).[3b)]

Adriamycin has been used extensively in the treatment of a variety of malignant diseases. However, the clinical usefulness of this anticancer agent is severely restricted by the high toxicity.[4)] Any dosage form or derivative of adriamycin with a lesser degree of toxicity or higher chemotherapeutic efficiency should be of great interest in the treatment of the various forms of leukemia and solid tumors.

The present investigation was therefore undertaken to evaluate the antitumor activity of fibrinogen microparticles containing adriamycin against Ehrlich ascites carcinoma in mice.

Experimental

Materials——Adriamycin hydrochloride was generously supplied by Kyowa Hakko Kogyo Co., Tokyo. Fibrinogen from bovine blood (Type I-S) was purchased from Sigma Chemical Co., St. Louis and used without further purification.

Preparation of Fibrinogen Microparticles——Fibrinogen microparticles containing adriamycin were prepared based on the same principle as used for the preparation of albumin microspheres.[3,5)] Adriamycin hydrochloride (30 mg) and bovine blood fibrinogen (100 mg) were dissolved in 1 ml each of distilled water. The drug and fibrinogen solutions were combined. The resulting solution was mixed with 100 ml of 10% Span 85 in cottonseed oil, and homogenized (Nihon Seiki Seisakusho, type HB) for 10 min at 4500 rpm. The emulsion was added to an additional 100 ml of cottonseed oil preheated to 90 °C and, after standing for 30 min with constant stirring, cooled to room

temperature. The microparticles were washed free of oil by adding 200 ml of ether, centrifuging for 10 min at 4500 rpm, and decanting the supernate. After the third wash, the microparticles were allowed to dry in a desiccator.

Microscopic Characterization of Microparticles——The dried microparticles were observed with a scanning electron microscope (Hitachi Seisakusho, type X-650). Photographs were taken after metal coating with an ion-coater (Eiko Seiki, model IB-3).

Drug Contents in Microparticles——The amounts of adriamycin in the final products were determined based on the method of Widder *et al.*[6]

Measurement of Release Rate——Drug release from the fibrinogen microparticles was determined as described previously,[7] using plastic dialysis cells with a cellulose membrane (Visking Co., type 36/32). Data shown in Fig. 2 are averages of three experimental runs and the results were satisfactorily reproducible.

Animal Experiments——Male ddY mice, 27—34 g, were used. For evaluating the antitumor effect of the fibrinogen microparticles, ddY mice were inoculated intraperitoneally with 2×10^6 Ehrlich ascites carcinoma cells. One day after inoculation of the cells, the mice were injected with a suspension of the fibrinogen microparticles containing adriamycin or with adriamycin solution (free adriamycin) in 0.9% NaCl containing 0.2% (v/v) Tween 80. Survival times of the tumor-bearing mice were recorded. The mice were observed for 60 d.

Toxicity Studies——The toxicity of the fibrinogen microparticles containing adriamycin was evaluated in terms of survivors on day 35 after intraperitoneal single administration to normal ddY mice.

Results and Discussion

Characteristics and Drug Contents of Fibrinogen Microparticles

Figure 1 (A) shows a scanning electron micrograph of the fibrinogen microparticles containing adriamycin prepared at 90 °C. They were irregular particles with a rough surface. On the other hand, the shape of fibrinogen microspheres containing adriamycin prepared at 140 °C was invariably spherical with a smooth surface.[3b] Fibrinogen microspheres containing 5-fluorouracil were obtained at the lower temperature (90 °C) of microsphere preparation.[3a] The development of a suitable method for the preparation of fibrinogen microspheres containing drugs requires a knowledge of the physicochemical properties of the agents. The stability of drug–fibrinogen aqueous solution in cottonseed oil emulsions and the shape of fibrinogen microspheres might be influenced by the viscosity of the mixed aqueous solution at the first step of the preparation of microspheres, and this in turn might depend on differences

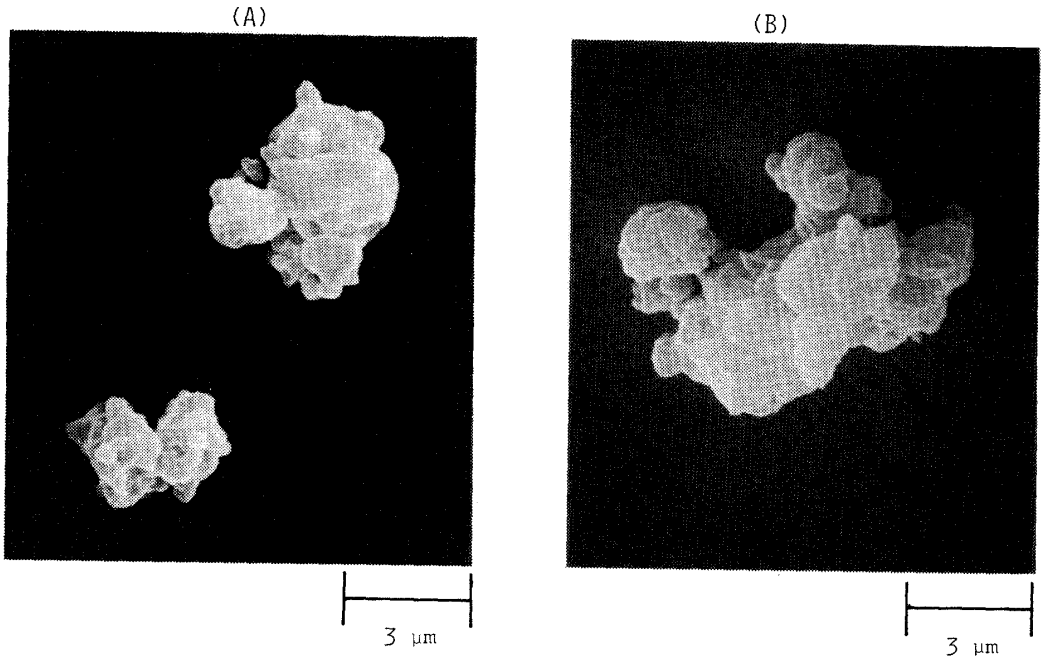

Fig. 1. Scanning Electron Micrographs of Fibrinogen Microparticles Containing Adriamycin, (A) before Release, (B) after Release for 7 d

of physicochemical interactions between fibrinogen and the drugs. The temperature of heat denaturation will also affect the shape of the microspheres.

The average diameter of the present fibrinogen microparticles was $2.4 \pm 0.2\,\mu m$ (mean $\pm$ S.E., $n=50$) as measured in photomicrographs. The average yield of fibrinogen microparticles from the preparation was $64.4 \pm 2.1\%$ (mean $\pm$ S.E., $n=13$) and the percent of adriamycin entrapped in the microparticles was 10.5 ± 0.9 (mean $\pm$ S.E., $n=3$).

In Vitro Release of Adriamycin from Fibrinogen Microparticles

The amount of adriamycin which can be released from fibrinogen microparticles into 0.9% NaCl was determined with a dialysis cell as described earlier.[7] Figure 2 shows plots of the data, expressed as the cumulative amount of the drug released *versus* time. In contrast with the rapid release of free adriamycin, the release of the drug from microparticles through the membrane was slow, indicating that sustained release occurs. Entrapment in the fibrinogen microparticles resulted in a remarkable retardation of the release of adriamycin.

A scanning electron micrograph showed that fibrinogen microparticles after drug release for 7 d were aggregated (Fig. 1(B)). Such aggregation of the fibrinogen microparticles was considered to be largely a result of the temperature of incubation (37 °C). Adriamycin was considered to have been released mainly by diffusion through the polymer matrix, because the surfaces of the microparticles after the release experiments had not changed significantly.

Antitumor Activity of the Fibrinogen Microparticles Containing Adriamycin against Ehrlich Ascites Carcinoma

The antitumor activity of fibrinogen microparticles containing adriamycin was evaluated against Ehrlich ascites carcinoma in mice. Tumor cell injections were made on day 0 and microparticle injections on day 1, both intraperitoneally.

Figure 3 shows the antitumor activity of fibrinogen microparticles containing adriamycin in comparison with that of free adriamycin. Four dose levels (6.9, 10.3, 13.7, and 17.1 mg/kg) were used in this study. When 2×10^6 cells of Ehrlich ascites were inoculated intraperitoneally into mice, the animals died between 17 and 25 d later due to the carcinoma (control in Fig. 3), and the mean survival time was 20.1 ± 0.8 d (mean $\pm$ S.E., $n=12$). Injection of fibrinogen microparticles without drug did not produce any significant prolongation of survival time (Fig. 3(B)). As is apparent in Fig. 3, free adriamycin exhibited maximum antitumor activities on Ehrlich ascites carcinoma at a dose of 6.9 mg/kg. Above this dose, free adriamycin exhibited severe toxicity. On the other hand, fibrinogen microparticles had almost the same activity as free adriamycin at a dose of 6.9 mg/kg, but above this dose, the microparticles

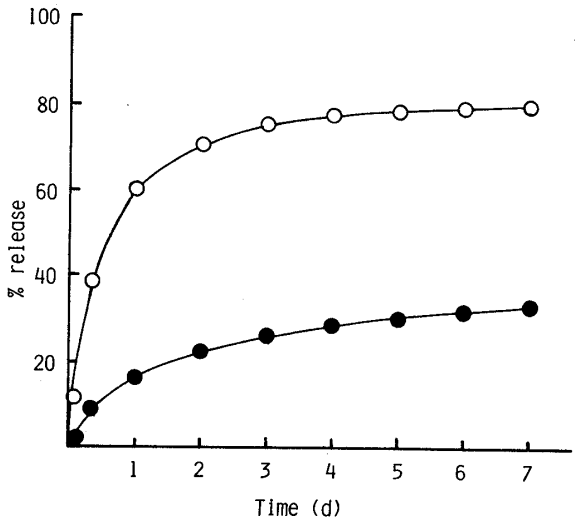

Fig. 2. Release Patterns of Adriamycin from Fibrinogen Microparticles at 37 °C

○, free adriamycin; ●, microparticles containing adriamycin.

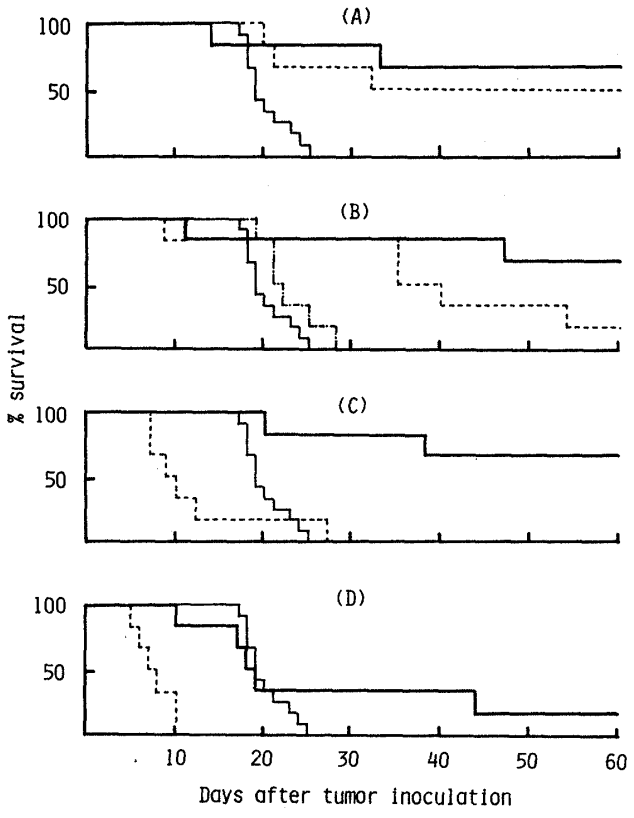

Fig. 3. Effect of Fibrinogen Microparticles Containing Adriamycin and Free Adriamycin (6.9 (A), 10.3 (B), 13.7 (C), and 17.1 (D) mg/kg) on the Survival Time of Mice Bearing Ehrlich Ascites Carcinoma

——, control; ------, free adriamycin; — - —, microparticles without drug; ——, microparticles containing adriamycin.

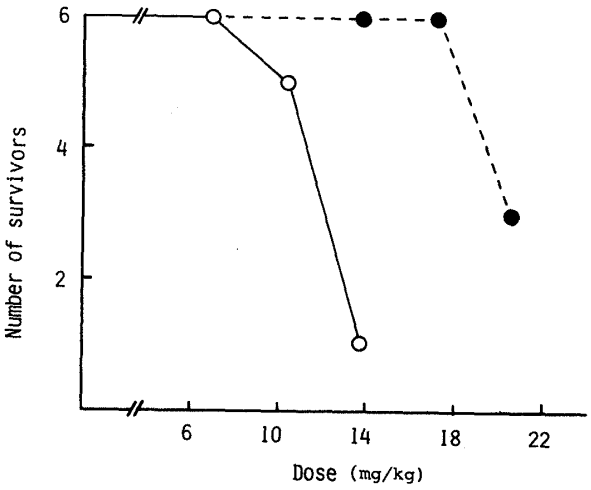

Fig. 4. Toxicity of free Adriamycin and Fibrinogen Microparticles Containing Adriamycin in Normal Mice

○, free adriamycin; ●, microparticles containing adriamycin.

containing adriamycin were therapeutically more active than adriamycin alone.

These results indicate that sustained release occurs in the peritoneum and that effective drug concentrations may be maintained by injection of the fibrinogen microparticles. The high chemotherapeutic efficiency of fibrinogen–adriamycin microparticles is striking at high doses which would be toxic in the case of the free drug. This increased survival can be interpreted as a sign of reduced toxicity. The fibrinogen–adriamycin microparticles appear to be less toxic than the equivalent dose of free adriamycin. LD_{50} of free adriamycin in male mice is estimated to be 13.7 mg/kg,[8] but may be considerably higher when adriamycin is administered in the form of fibrinogen microparticles containing the drug. It is possible that the required total doses as well as the systemic side effects of adriamycin might be significantly reduced by using this delivery system.

Toxicity to Mice

Figure 4 shows the 35 d survival data after a single injection of free adriamycin or adriamycin–fibrinogen microparticles into normal ddY mice. For the same amount of adriamycin, survival was much increased in the microparticle–adriamycin group. None of the 6 mice examined died even at the high dosages tested (17.1 and 20.6 mg/kg). This increased survival was interpreted as a sign of reduced toxicity. This difference in toxicity between the free and microparticle form of adriamycin corresponded to increased therapeutic effectiveness as evaluated in terms of survival data (Fig. 3).

These results suggest that injection of sustained-release fibrinogen microparticles containing adriamycin may be an effective means of cancer chemotherapy, providing a convenient method of drug delivery to target tissues while minimizing drug toxicity and maximizing drug effectiveness. Fibrinogen microparticles should be useful as a novel drug carrier for injectable delivery systems for anticancer agents.

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References and Notes

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