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Antitumour effect of fibrinogen microspheres containing doxorubicin on Ehrlich ascites carcinoma

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The antitumour activity of fibrinogen microspheres containing doxorubicin has been evaluated against Ehrlich ascites carcinoma in mice in terms of changes in body weight and survival. Tumour cell injections were made on day 0 and microsphere injections on day 1, both intraperitoneally. The suppressive effect of the drug-containing microspheres on increase in body weight was higher than that of the free drug, and tumour-bearing mice given the microspheres lived longer than those given the free drug.

Attempts have been made to deliver antitumour drugs to target sites within the tumour by means of drug delivery systems. The use of microspheres as sustained release targeting agents for antitumour drugs has received much attention. Albumin (Kramer 1974), gelatin (Yoshioka et al 1981), and polylactic acid (Juni et al 1985) have been investigated for use in drug delivery systems for antitumour drugs.

The possible use of fibrin film, a bioplastic prepared from human plasma, as a biodegradable carrier for drug delivery systems has been examined (Miyazaki & Nadai 1980; Miyazaki et al 1982). In addition to fibrin, the drug-carrier properties of fibrinogen in chemotherapy also appeared to be of interest and its potential use as microspheres forming an injectable, biodegradable system for the sustained release of drugs has been investigated. Fibrinogen, the precursor of fibrin, is a large, highly elongated, asymmetric molecule with a weight of approximately 340 000; it is usually used as a coagulant and rarely as a carrier of antitumour drugs (Szekerke et al 1972). Injectable microspheres prepared from fibrinogen should yield a novel biodegradable device from drug delivery.

This preliminary report describes the preparation, release characteristics in-vitro, and evaluation of antitumour activities of fibrinogen microspheres containing doxorubicin.

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Materials and methods

Materials. Doxorubicin hydrochloride (Adriamycin) 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 the fibrinogen microspheres. Fibrinogen microspheres containing doxorubicin were prepared on the same principle as albumin microspheres (Scheffil et al 1972). Doxorubicin hydrochloride (30 mg) and bovine blood fibrinogen (100 mg) were each dissolved in 1 ml of distilled water and the solutions combined and mixed with 100 ml of 10% Span 85 in cottonseed oil, and homogenized (Nihonseiki Seisakusho, Type HB) for 10 min at 4500 rev min^{-1}. The emulsion was added to an additional 100 ml of cottonseed oil preheated to a desired temperature (140 °C), allowed to stand for 30 min with constant stirring, and then cooled to room temperature (20 °C). The microspheres were washed free of oil by adding 200 ml of ether, centrifuging for 10 min at 4500 rev min^{-1}, and decanting the supernatant. After the third wash, the microspheres were allowed to dry in a desiccator.

Measurement of release rate. Drug release from the fibrinogen microspheres was determined as described by Miyazaki & Nadai (1980), plastic dialysis cells with a cellulose membrane (Visking Co., Type 36/32) being used. The capacity of each half cell was 4 ml and the surface area of the membrane was 3·14 cm^2. Fibrinogen microspheres containing 1 mg of doxorubicin were suspended in 4 ml of 0·9% NaCl and the suspension placed in the donor compartment. An equal volume of 0·9% NaCl was placed in the receptor compartment. The assembled cell was shaken horizontally at the rate of 100 strokes min^{-1} in an incubator maintained at 37 °C. The total volume of the receptor solution was

removed at intervals and replaced by 4 ml of the fresh release medium. The drug concentration of the samples was determined at 233 nm. Release studies were made in triplicate and the average values were plotted.

Animal experiment. Male ddY mice, 26–34 g, were used to evaluate the antitumour effect of the fibrinogen microspheres. They were inoculated intraperitoneally with 2×10^6 Ehrlich ascites carcinoma cells. One day after inoculation of the cells, the mice were injected either with a suspension of the fibrinogen microspheres containing doxorubicin or doxorubicin (free drug) in 0·9% NaCl containing 0·2% (v/v) polysorbate 80. Changes in body weight and survival time of the mice were recorded for up to 60 days.

Results and discussion

The dried microspheres were examined by scanning electron microscopy. Fig. 1A shows micrographs of the fibrinogen microspheres containing doxorubicin prepared at 140 °C. The microspheres were generally spherical with an average diameter of 1·7 ± 0·7 μm (mean ± s.d., n = 400) as measured in photomicrographs.

The determination of the amounts of doxorubicin embedded in the final products was based on the method of Widder et al (1979). When the fibrinogen microspheres were prepared by the method described, 9·9 ± 0·6% (mean ± s.e., n = 4) of drug in the original emulsion was associated with the microspheres.

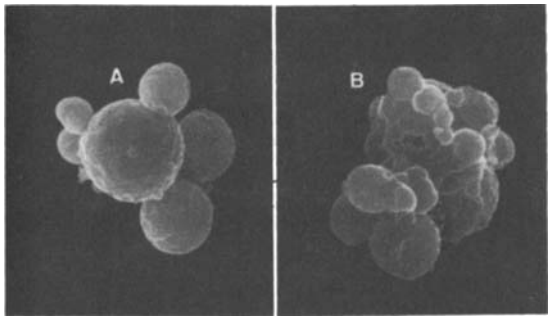

FIG. 1. Scanning electron micrographs of fibrinogen microspheres containing doxorubicin, (A) before release, (B) after release for 7 days. Scale: 1 cm = 3 μm.

The amount of the drug which can be released from fibrinogen microspheres into 0·9% NaCl was determined with a dialysis cell (Miyazaki & Nadai 1980). Fig. 2 shows plots of the data, expressed as the cumulative amount of drug released versus time. In contrast with rapid release of free doxorubicin from cellulose membranes, its release rate from microspheres through the membrane was slow, indicating that sustained release occurs. Thus, entrapment in the fibrinogen microspheres resulted in retarded release of the drug. A scanning electron micrograph showing changes in the

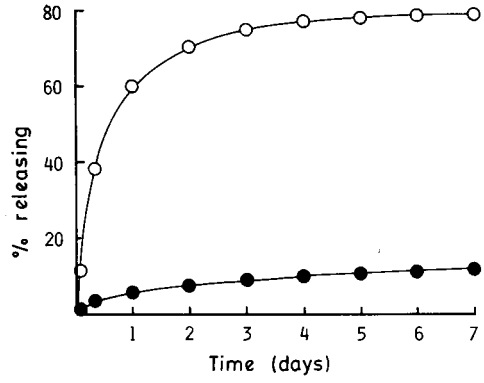

FIG. 2. Release patterns of doxorubicin from fibrinogen microspheres at 37 °C: ○, free drug; ●, microspheres.

surface characteristics of microspheres with time revealed that their surface before release was round and relatively smooth (Fig. 1A), but with time the surface became rough and the shape tended to be non-spherical after 7 days (Fig. 1B). The microspheres also formed aggregates. It seems likely that the mode of drug release from the microspheres is mainly one of diffusion.

Doxorubicin has been used extensively in the treatment of a variety of malignant diseases. However, its clinical usefulness is severely restricted by its high toxicity. Any dosage form or derivative of it with less toxicity or higher chemotherapeutic efficiency should therefore be considered. The antitumour activity of fibrinogen microspheres containing the drug was therefore evaluated against Ehrlich ascites carcinoma in mice.

The antitumour effect of the microspheres was evaluated by following changes in body weight of mice given the microspheres in amounts equivalent to 13·7 or 34·3 mg kg^{-1} of doxorubicin and in mice treated with the same doses of free doxorubicin. Fig. 3 shows the results from normal mice and from tumour-bearing mice so treated. At both doses, the increase in body weight after injection of the microspheres was less than that in untreated tumour-bearing mice while the mice receiving either dose of the free drug showed a fall in body weight relative to the control animals due to the toxicity of the drug.

The antitumour effect of the microspheres was also evaluated in terms of animal survival data (Table 1) against Ehrlich ascites carcinoma in comparison with that of free drug. Microspheres containing 13·7 or 34·3 mg kg^{-1} of the drug increased the T/C ratios to 179·6 and 191·3%, respectively, whereas free drug showed marked toxicity at both doses. One of six mice survived over 60 days after injection of fibrinogen microspheres containing doxorubicin.

As the microspheres containing doxorubicin limited the increase in body weight due to tumour growth (Fig. 3) and prolonged the life of the carcinoma-bearing mice

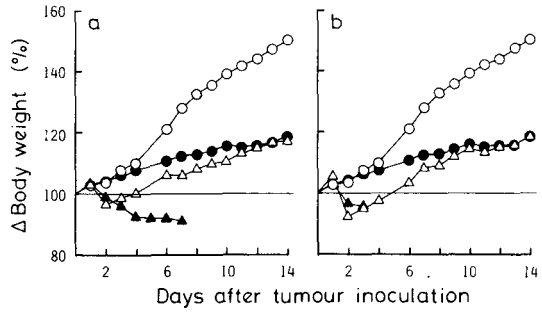

FIG. 3. Changes in body weight of Ehrlich ascites tumour-bearing mice after injection of fibrinogen microspheres containing (a) 13·7 and (b) 34·3 mg kg^{-1} of doxorubicin: ●, control normal mice; ○, control tumour-bearing mice; ▲, treated with free drug; △, treated with fibrinogen microspheres containing doxorubicin.

Table 1. Effect of single doses of fibrinogen microspheres containing doxorubicin or free drug in solution on survival time of ddY mice inoculated intraperitoneally with Ehrlich ascites carcinoma (2×10^6 cells).

Compound	Dose (mg kg^{-1})	Mean survival time (days) ± s.e.	T/C[a] (%)	Survivors at 60 days
Control	—	20·6 ± 0·7	100·0	0/18
Free drug	13·7	12·0 ± 3·1	58·3	0/6
	34·3	3·3 ± 0·3	16·0	0/6
Fibrinogen microspheres containing drug	13·7	37·0 ± 2·2*	179·6	1/6
	34 3	39·4 ± 5·5**	191·3	1/6

[a] Calculated as the ratio of the mean survival time of the treated group divided by that of the control group.
* Significantly different ($P < 0·005$) from the control.
** Significantly different ($P < 0·05$) from the control.

(Table 1), it may be inferred that sustained release occurs in the peritoneum and that effective drug concentrations may be maintained by the preparation. Their high chemotherapeutic efficiency contrasts with the toxic effect of the same doses given as free drug (Fig. 3). (The LD50 of free drug in mice is estimated to be 3–7 mg kg^{-1}, Oguro et al 1973).

Fibrinogen microspheres were prepared without drug and were evaluated in terms of the number of survivors and evidence of rejection 30 days after intraperitoneal injection into normal ddY mice. None of the 6 mice receiving the high dosage (50 mg microspheres per mouse) died and no localized inflammation or foreign reaction in the peritoneum was observed. The changes in body weight after injection of the microspheres were similar to those of normal untreated mice. Thus, fibrinogen microspheres showed biocompatibility. The toxicity of the microspheres containing doxorubicin was also evaluated for 35 days after a single intraperitoneal administration to normal ddY mice. None of the 6 mice examined died even at the highest dosage tested (68 mg kg^{-1}).

These results suggest that injection of sustained release fibrinogen microspheres containing doxorubicin has potential as a method of drug delivery which minimizes drug toxicity and maximizes drug effectiveness.

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