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Albumin microspheres as a drug delivery system for epirubicin: pharmaceutical, pharmacokinetic and biological aspects

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Summary

The aim of the present work was to investigate the effectiveness of epirubicin-loaded microspheres to affect specific organ or tissue sites where tumors are present. Egg albumin microspheres were prepared using a heat denaturation method. The kinetics of in vitro release of the drug complied partially with first order ($r = 0.9894$) as well as with diffusion model from a matrix ($r = 0.9982$). The pharmacokinetics of epirubicin in serum, heart and lungs after a single i.v. dose of free epirubicin and epirubicin-loaded microspheres in rats could be described by the equation of the two-compartment open model. The pharmacokinetic parameters calculated suggest the possibility to improve the selective entrapment of epirubicin-loaded microspheres by the lungs. The aim of our efficacy studies was to evaluate intratumoral administration of epirubicin-loaded microspheres in mice with Ehrlich ascites carcinoma, as well as experimental metastases in 256 rats with Walker carcinoma. The difference between the mean survival time, respectively the final metastatic incidences of the animals from treated groups compared to controls and to the free-epirubicin group, were statistically significant. These findings suggest the possibility of organ targeting and of reducing unwanted side-effects, especially the cardiotoxicity, of the epirubicin-loaded microspheres in cancer chemotherapy.

Introduction

The use of particulate microspheres is perhaps one of the most promising systems of drug delivery to specific organs in the body. A major aim in cancer chemotherapy is to improve the efficiency of the cytostatic treatment. The killing of the sensitive tumor cells will be facilitated by exposing the cancerous lesions to high concentrations of anticancer drug.

Anthracycline derivatives like epirubicin are potent antitumor agents. They are widely used in cancer chemotherapy against a broad spectrum of tumors. However, by the conventional administration it is not possible to use very high doses of drugs due to their secondary toxic effects. Therefore, in order to improve the accumulation of cytostatic drugs at the tumor level, different experimental drug delivery systems, such as liposomes, microspheres, emulsions, nanoparticles, were realised (Davis et al., 1984; Weinstein, 1984; Willmott et al., 1985; Mizushima, 1985; Oppenheim, 1981; Leucuţa, 1986).

The purpose of our study was: (a) to prepare egg albumin microspheres as a sustained release targeting system for the antitumor drug epirubicin; (b) to evaluate the pharmacokinetics of the drug after i.v. administration of the microspheres

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in rats, compared with the drug alone; and (c) to evaluate the development of Ehrlich ascites carcinoma in mice intratumorally treated by injection of albumin microspheres containing epirubicin, as well as to study the evolution of the experimental metastases (256 Walker carcinomas) in rats treated with epirubicin-loaded microspheres.

Materials and Methods

Materials

The drug and additives used for the drug delivery system formulation were: epirubicin hydrochloride (Farmorubicin, Farmitalia, Carlo Erba), egg albumin (Fabrica de Praf de Ouă, Arad, Romania), Span 80 (Schuchard, Munchen), sunflower oil conform to the Romanian Pharmacopoea requirements.

Albumin microspheres preparation

Egg albumin microspheres containing epirubicin were prepared based on the principle of heat denaturation. Epirubicin hydrochloride (30 mg) and egg albumin (200 mg) were each dissolved in 1 ml distilled water. The drug and albumin solutions were combined. The resulting solution was mixed with 100 ml of 1% Span 80 sunflower oil and homogenized for 10 min at 2000 rpm. The emulsion was heated to 100 °C for 30 min under stirring, then cooled to room temperature. The microspheres formed were washed free of oil by ethyl ether, centrifugating for 5 min at 40000 rpm and decanting the supernate. The microspheres were allowed to dry in a desiccator.

Epirubicin content microspheres

A sample of epirubicin-loaded microspheres was accurately weighed, digested with trypsin and extracted into chloroform: isopropanol(2 : 1). After evaporation to dryness the sample was analysed by fluorimetry.

In vitro release of drug

Microspheres in aliquots of 10 mg were suspended in water at 37 °C with stirring (60 rpm) and at intervals aliquots were taken, centrifuged and the supernatants analysed for drug content. Release of epirubicin is expressed as the amount of drug in the supernatant as a percentage of drug in the aliquot of microspheres. The quantitation of epirubicin was made by UV spectrometry or by fluorimetry.

Animals

Female Wistar rats (180 ± 10 g) were used in the pharmacokinetic study. A dose of 10 mg/kg was i.v. administered to each animal as a microspheres dispersion in saline with 1% Tween 80.

Procedure

After i.v. administration of the drug, blood samples were obtained by exsanguination (following ether anesthesia) at different intervals (5; 15; 30 min; 1, 2, 4, 6, 24 and 48 h). Lungs and heart were removed at the same intervals. Serum and organ homogenates were extracted into chloroform : isopropanol (2 : 1). After evaporation to dryness samples were analysed by fluorimetry.

Pharmacokinetic analysis

The serum or organ concentration–time curves were fitted to a sum of exponentials using a nonlinear least-square square regression fitting programme. The following pharmacokinetic constants were calculated: hybrid rate constant (α and β); distribution rate constants (k_{12} and k_{21}); biological half-life ($t_{1/2}$); apparent volume of distribution ($V_{d,area}$); area under the curve from time $t = 0$ to $t = \infty$ (AUC); body or organ clearance (Cl) (Leucuţa et al., 1981).

Study of therapeutic effects

The aim of our study was to evaluate the intratumoral administration and the experimental metastases.

Intratumoral administration. The experiments were performed on 98 Swiss female mice of 25 ± 0.75 g b.wt. The animals were grafted with 1×10^6 Ehrlich ascites carcinoma cells suspended in 0.5 ml saline. The groups were as follows: group I (20 mice), controls, untreated; group II (20 mice), treated with free epirubicin on the day of tumor inoculation (day 0); group III (19 mice), treated with albumin microspheres containing epirubicin, on the day of tumor inoculation (day 0); group IV (20 mice), treated with free epirubicin 7 days after

tumor inoculation (day +7); group V (19 mice) treated with epirubicin-loaded microspheres 7 days after tumor inoculation (day +7). In all treated groups the dose of epirubicin was 10 mg/kg b.wt. The animals were weighed twice a week. The survival of animals was registered dayly. The animals were followed for 150 days. The results were statistically analysed by the Student *t*-test.

Experimental metastases. The study of the effect of epirubicin-loaded microspheres on the evolution of the experimental metastases was performed on 78 Wistar rats (160 ± 20 g b.wt.). The animals were i.v. (tail vein) grafted with 2×10^6 Walker carcinoma cells. The animals were treated as follows: group I (30 rats), controls, untreated; group II (24 rats), treated with free epirubicin 7 days after tumor inoculation (day +7); group III (24 rats), treated with epirubicin loaded microspheres (day +7). The dose of epirubicin in each experiment was 10 mg/kg b.wt. The animals were followed for 70 days.

Results and Discussion

Pharmaceutical aspects

The microspheres were obtained as a free-flowing powder with a mean diameter of the beads of 20 μm and with a content of 12.5% epirubicin. The dissolution rate of epirubicin from the powder of microspheres is presented in Fig. 1. It can be seen that the release of epirubicin is virtually complete after 6–8 h. The kinetics of drug release is only partially conform to first order release ($r = 0.9894$), but it might conform to Higuchi's matrix dissolution model which offers a linear correlation between the cumulative percent of drug released as a function of the square root of time. However, a linear correlation only occurs between

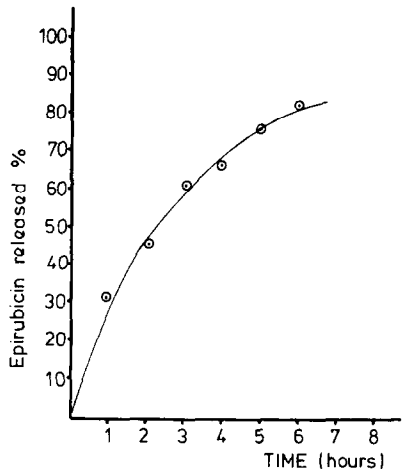

Fig. 1. Dissolution pattern of epirubicin from microspheres in water at 37 °C.

20%–70% release ($r = 0.9982$). The release of the drug was significantly delayed as compared to the powder of epirubicin which was almost instantaneously dissolved. The kinetics of release complied partially with first-order as well as with a diffusion model from a matrix, but a zero-order release has not been realised. It may well be that the two mechanisms are present but that one is predominate (Samuelov et al., 1979; Hecquet et al., 1984).

Pharmacokinetical aspects

The pharmacokinetic constants of epirubicin in serum and heart after a single i.v. dose of free epirubicin in rats are shown in Table 1. The pharmacokinetic parameters of epirubicin in serum, heart and lungs after a single i.v. dose of epirubicin-loaded microspheres in rats are presented in Table 2.

After a single i.v. dose of free epirubicin a

TABLE 1

Serum and heart pharmacokinetics of free epirubicin following i.v. administration of a single dose as aqueous solution to rats

	α (h^{-1})	β (h^{-1})	$t_{1/2}$ (h)	AUC ($\mu g \cdot h \cdot ml^{-1}$)	V_d (ml/kg)	Cl (ml/min)
Serum	-	0.7	1.0	10	1.9	100
Heart	3.3	0.4	1.1	20	0.12	50

TABLE 2

Serum, heart and lungs pharmacokinetics of epirubicin following administration of a single i.v. dose of epirubicin-loaded microspheres as aqueous dispersion to rats

	α (h^{-1})	β (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})	$t_{1/2}$ (h)	AUC ($\mu g \cdot h \cdot ml^{-1}$)	V_d (ml/kg)	Cl (ml/min)
Serum	0.7	0.05	0.18	0.5	14	25	0.9	40
Heart	0.8	0.2	0.3	0.4	3.5	12	0.4	83
Lungs	2.0	0.3	1.0	0.4	2.3	40	0.03	25

monoexponential equation adequately fitted to the data. After a single i.v. dose of epirubicin-loaded microspheres a biexponential equation was required to describe the data. The biological half-life in serum after free epirubicin administration was about 1 h, the AUC was half but the clearance was twice that observed in the heart. Pharmacokinetic studies with drug-loaded microspheres are lacking, but there are some studies with liposomes with similar results (Rosa et al., 1983).

I.v. delivery of microspheres with a diameter of about 20 μm leads to their mechanical filtration by the lungs (Tomlinson, 1983). As a consequence the pharmacokinetics of epirubicin incorporated in microspheres changed.

The half-life in the serum was prolonged to 14 h, whereas in the heart and lungs it was about 2–3 hours. On the other hand the clearance of epirubicin in serum was about half that in the heart and twice that in the lungs. The bioavailability in the lungs was twice that in the serum, whereas in the heart it was half that in the lungs.

These findings suggest the possibility to improve the selective antitumor activity using epirubicin-loaded microspheres. This reduces unwanted side-effects and adverse reactions, especially the cardiotoxicity, with a consequent increase in the therapeutic index.

Biological aspects

The evolution of the body weight in Ehrlich ascites carcinoma-bearing mice, controls and treated groups is presented in Fig. 2. The maximum weight growth of the tumor-bearing mice was reached in the control group (100%). At the same moment in the groups II, III, IV and V the gained weights were 15.6%, 25%, 18.7% and 52.5%

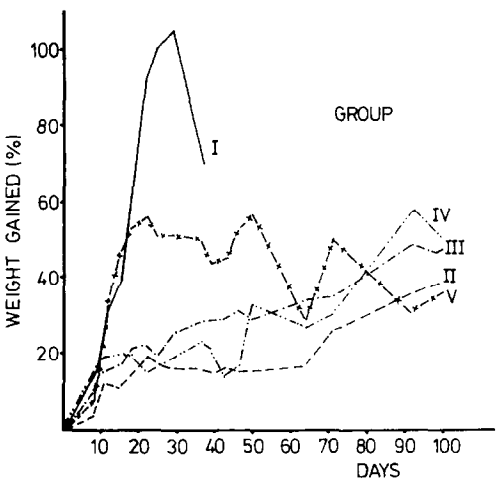

Fig. 2. Evolution of body weight in Ehrlich ascites carcinoma-bearing mice, controls and mice treated with epirubicin free and epirubicin-loaded microspheres.

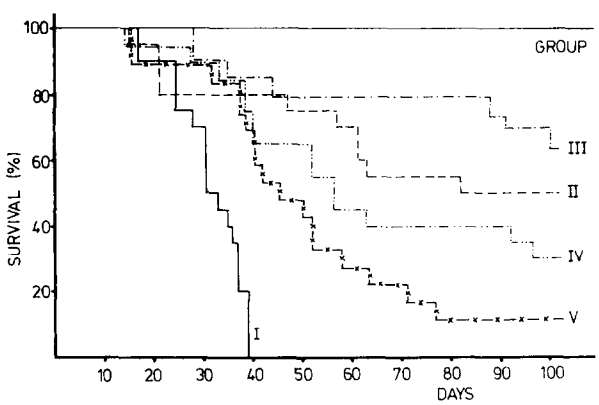

Fig. 3. The effect of free epirubicin and epirubicin-loaded microspheres on the survival of Ehrlich ascites carcinoma bearing mice.

respectively. The body weights of the survivors had values in the range of that in normal mice of the same age.

The treatment with free epirubicin and epirubicin-loaded microspheres increased the survival time in all treated groups (Fig. 3). In the group of animals treated with epirubicin-loaded microspheres on the day of tumor inoculation (day 0) the mean survival time had the highest value (116 $\pm$ 5 days) of all groups. The difference between the mean survival time of the controls and that of animals from groups II–IV was statistically significant ($P < 0.01$; $P < 0.01$; $P < 0.05$, respectively). The difference between the controls and treated groups V was not statistically significant.

In the 256 Walker carcinoma bearing rats, the final metastatic incidences were as follows: 73.3%, group I; 37.5%, group II; and 41.65%, group III. The difference between controls and treated groups was statistically significant ($P < 0.05$).

The antitumour effect of fibrinogen microspheres containing doxorubicin on Ehrlich ascites carcinoma was reported by Miyazaki et al. (1986). The entrapment of anthracyclines in systems which selectively and gradually release the drugs to the target organ or tissue sites where tumors are present has been shown to reduce their toxicity while maintaining their antitumor efficacy (Mayhew et al., 1983; Weiss et al., 1985).

Our results suggest the possibility of using epirubicin-loaded microspheres to specific target organ or tissue sites where tumors are present. At the same time this type of therapy could protect the host against the side effects of the cytotoxic agents. Further investigations are needed to demonstrate the efficiency of drug-loaded microspheres in cancer chemotherapy.

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