ONCOLOGY

Efficacy of Liposomal Forms of Cytostatics

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Study of the toxicity and pharmacokinetics of free and liposomal forms of cytostatics revealed an appreciable decrease of the toxicity of high drug doses and prolongation of drug action in liposomes. The distribution of drugs in organs and tissues is shown.

Key Words: *doxorubicin; fluorouracil; liposomes; toxicity; distribution*

The key to improving the efficacy of chemotherapy of malignant tumors lies in ensuring high drug concentrations in the vascular bed and the involved organ and in reducing the overall toxicity of the cytostatics used [1,13]. One possible solution is to use drugs encapsulated in liposomes [3-5,8]. However, the clinical use of liposomes requires a study of their properties, specifically, the optimal composition and method of their preparation, the toxicity and cytotoxicity of the drugs, the incorporation of the drugs in liposomes, and their subsequent distribution in the organism [3-7,13,14].

This paper presents the results of a laboratory study of the efficacy of liposomal forms of antineoplastic agents.

MATERIALS AND METHODS

Antitumor antibiotics of the anthracycline series were used in the study: doxorubicin (adriamycin) (DR, Moscow) or pharmorubicin (Farmitalia Carlo Erba) and carminomycin (CM, Moscow). In addition, the antimetabolite fluorouracil (FU, Kiev) was used. Liposomes were prepared from natural phospholipids isolated as described previously [2,12]. Sigma lipids and cytostatics were used as standards.

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Liposomes were obtained as described elsewhere [4,6,13] using sonication or dispersion on an α-Laval type high-pressure homogenizer. Liposome preparations were sterilized by filtration through Millipore membranes with 0.22 μ pores.

Lipid oxidation in liposomes was assessed by spectrophotometry [11]. Lipid chromatography was carried out in a thin silicagel layer on Merck plates in the chloroform:methanol:water (65:25:4) system. Lipid content was assessed from phosphorus [10]. The size of liposome particles was measured with a Cultronics autocorrelation spectrometer. The amount of cytostatic incorporated in the liposomes was assessed by gel filtration through Sephadex C-50 (Pharmacia). The sterility, pyrogenicity, and toxicity of the drugs were assessed using the methods recommended by the State Pharmacopoeia of the USSR.

The concentrations of antibiotics in the solution, blood serum, and tissue homogenates were measured on a Gilson chromatograph. Column: diasorb C-16, 150×4 mm (Elsiko, Moscow), precolumn C-18 (Merck). Detection by fluorescence, detector 121, sensitivity 0.1 RFU (relative fluorescence units), optical-interference filter: λ_{excit}=500±40 nm, $\lambda_{exp.}$=600±50 nm. Mobile phase: 1) eluent A - distilled water:methyl alcohol:10 mM solution of sodium hexysulfone in methyl alcohol:acetic acid (250:110:4:1); 2) eluent B - methyl alcohol:10 mM solution of sodium hexysulfone in methyl alcohol:acetic acid (360:5:1). Gradient for assessing DR: 1 min 0-

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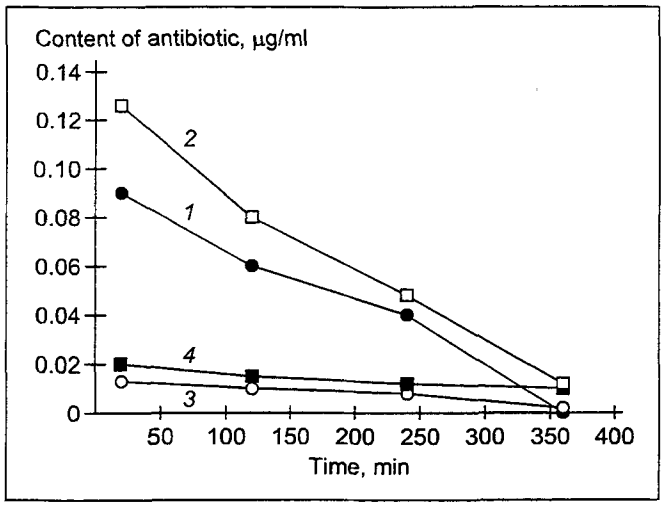

Fig. 1. Content of antibiotics in the blood of animals after injection of free and liposomal forms. DR: free (*1*) and liposomal (*2*) forms; CM: free (*3*) and liposomal (*4*) forms.

40%, 10 min 40-60%, 10.5 min 60-100% of eluent B. Gradient for assessing CM: 1 min 0-40%, 13 min 40-70%, 14 min 70-100% of eluent B.

The content of FU was measured in the solution, blood serum, and tissue homogenates using a Gilson chromatograph with a Rheodine 7125 injector. A 5-μl sample was introduced in the injector and analyzed on a Lichrosorb RP-18 3×150-mm column with a Lichrosorb RP-18 4×4-mm precolumn during elution with 1.25% methyl alcohol in 20 mM NaH_2PO_4, pH 5, at a flow rate of 1 ml/min and detection at 266 nm (0.01 AUFS - relative absorption units for the whole scale). The toxicity was assessed on outbred albino mice of both sexes weighing 20.0±0.2 g. Pyrogenicity and drug distribution in the organs were studied on rabbits of both sexes weighing 2.5 to 3.0 kg. The animals were fed standard diets.

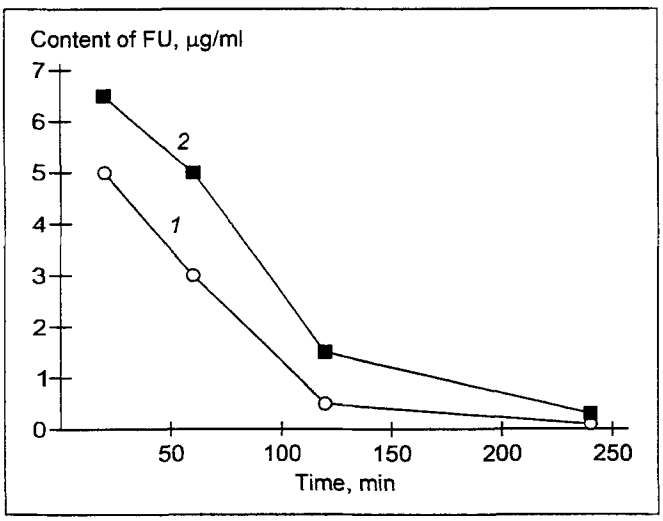

Fig. 2. Content of FU in the blood of animals after injection of the free (*1*) and liposomal (*2*) forms.

RESULTS

Both liquid and lyophilized preparations of liposomes were used in the study. Liposome size before lyophilization was 160±30 nm and after lyophilization 260±40 nm. Incorporation of cholesterol in the liposomes increased the size of particles obtained after lyophilization to 300±50 nm and reduced the amount of cytostatic binding to the liposomes. Incorporation of acid phospholipids, for example, glycerol diphosphate, in the liposomes improved the incorporation of anthracyclines by 8-12%. For DR the percentage of cytostatic incorporation in phosphatidylcholine liposomes was 78-85%, for CM 75-85%, and for FU 60-75%. We found cholesterol-containing liposomes to be less stable both in the course of preparation and during storage. The index of oxidation of the liposome preparations did not exceed 0.47-0.55.

In the first series of experiments we compared the distribution of antitumor antibiotics in the organs after their intravenous infusion in free and liposomal form. The drugs were injected to rabbits in a dose of 1 mg/kg. Drug accumulation in the serum, liver, spleen, brain, kidneys, lungs, and heart was assessed. The concentrations of the drugs in the blood after injection of their free forms were found to be appreciably lower than after injection of their liposomal forms (Fig. 1). For example, the content of liposomal DR 6 h after injection was 1.5-3 times higher than after injection of the free form. Moreover, incorporation of anthracyclines in liposomes prolongs their presence in the blood.

Measurement of the content of cytostatics in the myocardium revealed that DR bound to liposomes accumulated in the tissue in a concentration of 0.03-0.035 μg/g tissue 2 h after injection. On the other hand, the content of free DR was 2-2.3 times higher: 0.078-0.084 μg/g ($p<0.01$). Five hours after administration the content of the antibiotic injected in either form was virtually the same. No reliable difference was observed between the concentrations of liposomal and free CM in the heart. Injection of free DR and CM did not lead to the accumulation of these drugs in the brain, whereas liposome-bound forms of these antibiotics were detected in concentrations of 0.005 μg/g brain tissue for CM and 0.025-0.03 μg/g for DR ($p<0.01$). Apparently, liposome drugs of about 160 nm in size are capable of crossing the blood-brain barrier. The results of assessing antibiotic incorporation in the liver were somewhat unexpected. We found that liposome-bound cytostatics became incorporated in the liver less intensively than free drugs ($p<0.05$). The content of antibiotics in the spleen and kidneys virtually did not differ after injection of the two forms. This is confirmed by published data [9]. Cholesterol incorporation in the liposomes and the use of larger lipo-

TABLE 1. Toxicity of Free and Liposomal DR and CM

	Dose per mouse, μg (mg/kg)	Free form		Liposomal form	
		Number of mice: total/survivors	% survival	Number of mice: total/survivors	% survival
DR	100 (5)	14/12	85.71	14/14	100
	300 (15)	14/10	71.43	14/13	92.85
	400 (20)	14/2	14.28	12/4	33.3
	500 (25)	14/0	0	12/5	41.66
	600 (30)	13/0	0	14/0	0
CM	20 (1)	10/8	80.0	10/10	100
	100 (5)	12/2	16.6	10/6	60.0
	200 (10)	12/0	0	10/2	20.0
	300 (15)	12/0	0	10/0	0

somes (300 to 500 nm) markedly enhanced incorporation of the antibiotic by the reticuloendothelial system. In parallel with this, we discovered that CM is excreted from the organism much sooner.

The high cardiotoxicity of the above drugs and the lower accumulation of their liposomal forms in the myocardium prompted us to study the toxicity of free and liposomal DR and CM.

For this purpose, mice were intravenously injected various doses of the drugs and followed up for 10 days, with their body weight regularly measured. The results indicate (Table 1) that the liposomal forms of the antibiotics are less toxic than the free ones. CM is more toxic than DR. Incorporation of cholesterol, phosphatidylserine, and diphosphatidylglycerol in the liposomes did not reliably lower the toxicity of the antibiotics, especially of DR. On the other hand, incorporation of cholesterol (10-20%) in the liposomal CM increased animal mortality by 10-13%.

Hence, DR incorporated in phosphatidylcholine liposomes could be administered in a dose 1.5-2 times higher in comparison with its free form without its toxicity being altered.

In the second series of experiments we studied FU accumulation in the blood serum (Fig. 2) and organs (Table 2). One or two hours after injection of the liposomal form the content of FU in the serum was 1.4-2 times higher than after injection of its free form. Thereafter the drug concentration in the blood decreased. Administration of FU in the liposomal form prolonged its presence in the blood. Measurements of FU in the heart and bone marrow yielded particularly interesting results. One hour after injection of the liposomal form FU was found in the bone marrow in a concentration of 37.3±5.6 μg/g tissue. On the other hand, the content of FU after injection of the free form was much higher (86.9±9.0 μg/g tissue, $p<0.01$). The content of FU in the heart after injection of the free form was somewhat higher than after injection of the liposomal preparation ($p<0.05$). Hence, both types of cytostatics, FU and DR, show a lesser incorporation in the cardiac muscle after injection of the liposomal form. This is of special importance in view of their high cardiotoxicity. Study of the accumulation of the two drug forms in other organs showed no appreciable differences. Data on the toxicity of free and liposomal FU are presented in Table 2. The liposomal form of the agent proved to be less toxic than free FU. Study of FU distribution in tissues 4 h after injection revealed no differences in the drug levels.

The reasons why liposomes reduce the toxicity of cytostatics to such an extent may be related to the

TABLE 2. Toxicity of Free and Liposomal FU

	Dose per mouse, mg (mg/kg)	Number of mice: total/survivors	Mouse weight, g	Time of death, day	% survival
Free form	1.5 (75)	12/9	20.6	6	75
	2.0 (100)	12/9	19.8	5	75
	2.5 (125)	12/4	18.0	5	33
	3.0 (150)	12/0	-	4	0
	3.5 (175)	14/0	-	4	0
	4.0 (200)	10/0	-	4	0
Liposomal form	1.5 (75)	10/9	21.3	3	90
	2.0 (100)	10/10	21.4	-	100
	2.5 (125)	12/10	20.0	10	83
	3.0 (150)	14/7	19.6	9	50
	3.5 (175)	12/2	17.8	9	16
	4.0 (200)	10/0	-	7	0

altered pharmacokinetics of the drugs, namely, to differences in the drug distribution in organs and tissues.

Hence, the use of liposomal forms of cytostatics boosts their specific action by depositing the drugs in the blood and, hence, prolonging their effect on the tissues and evidently on the tumor. Along with this, the content of cytostatics in some organs decreases and thus their toxicity for healthy tissues is appreciably reduced. These results give the green light for a clinical study of the two forms of cytostatics.

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