ANTITUMOR ACTIVITY OF LIPOSOMAL ACLARUBICIN IN VITRO AND IN VIVO

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Liposomes are a promising therapeutic form of certain biologically active substances (cytostatics, immunomodulators, antibiotics, etc., totaling more than 100 therapeutic preparations) [5, 6, 10]. A problem of particular importance is how to prepare liposomal forms of antitumor agents that exhibit insufficient selectivity of action and are characterized by a low chemotherapeutic index. Liposomal forms of various antitumor preparations, including doxorubicin, daunorubicin, etc., have so far been developed [3, 6]. By incorporating cytostatics into liposomes their chemotherapeutic properties can be improved, and in some cases delivery of the preparations can be targeted on particular organs (the liver, spleen, or lungs); in this case the principal target cells for liposomal preparations are cells of the mononuclear phagocytic system [7-9]. Accordingly, the creation of liposomal forms of preparations which also have the property of activating macrophages is a particularly interesting question. These cytostatics include the antitumor anthracycline antibiotic aclarubicin (Acr), which exhibits immunomodulating activity and, in particular, it induces tumoricidal properties in human and murine peritoneal macrophages (Mph) [2-4].

The aim of the present investigation was to study the antitumor activity of the liposomal form of Acr in vitro and in vivo.

EXPERIMENTAL METHOD

Acr was obtained from the All-Union Antibiotics Research Center, Moscow. The liposomal form of Acr (Lp-Acr) was prepared by continuous-flow detergent dialysis, with the aid of the "Liposomat" apparatus ("Dianorma," West Germany) [1]. The liposomal emulsion contained unilamellar liposomes measuring from 60 to 120 nm. The structure-forming raw materials were egg phosphatidylcholine, cholesterol (purified five times over), and stearic acid (all of Soviet origin), in the molar ratio of 10:0.3:0.17. The quantity of Acr incorporated into the liposomes was 82-93%; the Acr concentration in the liposomal emulsion was 800-1000 μg/ml and in lipids it was 25-30 mg/ml. The cytostatic action of Acr and Lp-Acr was estimated on the basis of inhibition of incorporation of ^{3}H-thymidine into cells of murine lymphatic leukemia P388 [4], by sampling on a 12-channel harvester ("Dynatech") and counting radioactivity on a "Mark III" beta scintillation counter. The therapeutic action of the preparations was estimated in albino mice with an Ehrlich's ascites carcinoma, with respect to inhibition of ascites formation; antimetastatic activity was studied on a model of "artificial" lung metastases of a B16 melanoma in syngeneic C57BL/6 mice, and toxicity was studied in albino mice with Ehrlich's ascites carcinoma on the basis of death of the animals without ascites during the 7-day period of observation. The results were subjected to statistical analysis by Student's t-test and also by computer programs for calculating IC_{50} and IC_{90} (the concentrations of the preparation inhibiting incorporation of ^{3}H-thymidine by 50 and 90% respectively), TD_{50}, and LD_{50} (doses of the preparations leading to a 50% therapeutic effect or to 50% mortality among the animals respectively).

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TABLE 1. Dependence of Cytostatic Action of Liposomal and Free Acr on P388 Cells on Presence of Mph

Form of Acr	Mph	IC_{90}, μg/ml	
		duration of incubation, h	
		24	48
Free	—	0,3±0,037	0,13±0,06
Free	+	0,066±0,012	0,037±0,009
Liposomal	—	1,12±0,28	0,35±0,07
Liposomal	+	0,065±0,01	0,00082±0.0002

Legend. P388 cells ($2 \cdot 10^4$/well/0.2 ml) incubated for 24 or 48 h with free Acr or Lp-Acr in presence of murine peritoneal Mph ($4 \cdot 10^4$/well) or without Mph; control – P388 cells incubated in absence of Acr, with or without Mph.

TABLE 2. Therapeutic Action of Liposomal and Free Acr on Ehrlich's Ascites Carcinoma in Albino Mice

Form of Acr	Dose, mg/kg	Number of mice without ascites, %	Inhibition of growth of ascites tumor, %	Number of mice dying without ascites during 10 days of obs.	Changes in body weight, %	Changes in weight of spleen, %
Free	25	100	100	50	—30	—86
	12,5	100	100	25	—22	—51
	5	50	60	0	—9	—14
	2,5	0	18	0	—4	—14
Liposomal	25	100	100	0	—17	—29
	12,5	33	75	0	—4	0
	5	0	20	0	—2	0
	2,5	0	16	0	0	0

TABLE 3. Antimetastatic Action of Liposomal and Free Acr

Preparation	Number of lung metastases per mouse	Number of mice without metastases, %
Control	39,1±6,0	0
Free Acr	11,9±3,8* (70)	0
Liposomal Acr	5,3±0,6* (86)	50

Legend. C57BL/6 mice were given intraperitoneal injection of $2 \cdot 10^5$ melanoma B16 cells. Preparations were injected intravenously in a dose of 5 mg/kg (as Acr) on the 1st, 5th, and 9th days. Number of lung metastases were counted on 21st day. Percentage inhibition compared with control shown in parentheses. *) Significance of difference of parameters between groups ($p < 0.05$).

EXPERIMENTAL RESULTS

The in vitro study of the cytostatic action of Acr and Lp-Acr against P388 cells showed that the cytotoxicity of Lp-Acr, as a rule, was lower than that of the original antibiotic. For instance, IC_{50} of Acr was 0.018 ± 0.00053 μg/ml, and IC_{50} of various samples of Lp-Acr varied from 0.03 ± 0.001 to 0.05 ± 0.0027 μg/ml (differences statistically significant).

In the presence of murine peritoneal Mph the cytostatic action of Lp-Acr was greatly intensified. The cytotoxicity of the Acr itself also was increased in the presence of Mph, but by a much lesser degree than the cytotoxicity of Lp-Acr; the duration of incubation of the preparations with the tumor cells was a matter of great importance in this respect (Table 1). As Table 1 shows, in the presence of Mph, IC_{90} of Lp-Acr after 24 h of incubation with tumor cells fell by 17.2 times, and after 48 h it had fallen by 427 times. In the presence of Mph the value of IC_{90} of free Acr also decreased, but by a much lesser degree (by 4.5 and 3.5 times respectively). Incidentally, whereas after incubation for 24 h the cytostatic activity of the two forms of the antibiotic in the presence of Mph was virtually identical, after 48 h the cytotoxic action of Lp-Acr was much (45 times) greater than that of the free preparation. The results indicate a much stronger activating effect of Lp-Acr on Mph than that of the free antibiotic, probably due to gradual release of the preparation from lipid vesicles phagocytosed by Mph. Incidentally Lp-Acr is probably an attractive stimulus for Mph. For instance, on the 4th day after intraperitoneal injection of Lp-Acr (2.5 mg/kg as Acr) from BDF1 mice, twice as many peritoneal Mph could be isolated [$(4.6 \pm 1.5) \cdot 10^6$/mouse] than after injection of the equivalent quantities of Acr [$(2.6 \pm 0.5) \cdot 10^6$/mouse], or of empty liposomes [$(2.8 \pm 0.8) \cdot 10^6$/mouse] or from intact mice [$(2.4 \pm 1.0) \cdot 10^6$/mouse].

The study of the therapeutic action of the two forms of the antibiotic against Ehrlich's ascites carcinoma showed that the antitumor activity of Lp-Acr in high doses (25 mg/kg, on the 1st day, intraperitoneally) was comparable with activity of the original preparation (Table 2). In a dose of 12.5 mg/kg Acr led to similar inhibition of ascites formation in 100% of cases, whereas Lp-Acr gave the same effect in only 33% of animals. Meanwhile, if Acr was given in doses of 12.5 or 25 mg/kg death of 25 and 50% of the mice respectively was observed, whereas Lp-Acr had no toxic action even in a dose of 30 mg/kg (these last data are not shown in Table 2). Differences in the toxicity of the two forms of Acr also were exhibited when body weight parameters and the weight of the animals' spleen were estimated (Table 2). In mice with Ehrlich's ascites carcinoma TD_{50} of Acr was 4.04 ± 0.55 mg/kg, LD50 was 18.71 (14.1-24.83) mg/kg, and the chemotherapeutic index (LD_{50}/TD_{50}) was 4.63 ± 0.36. Meanwhile TD_{50} of Lp-Acr was 6.11 ± 0.96 mg/kg, whereas its LD_{50} was considerably above 30 mg/kg (the largest dose used which did not cause death of the animals), evidence that the chemotherapeutic index of Lp-Acr (>4.91) is significantly higher than that of the free antibiotic. Empty liposomes not only had no antitumor action, but they actually led to marked stimulation (by 78%) of ascites formation.

Lp-Acr had a more marked metastatic action (on a model of "artificial" metastases of melanoma B16) than the original preparation (Table 3). In 50% of mice receiving Lp-Acr complete suppression of formation of lung metastases was observed. The great importance of natural mechanisms of antitumor surveillance (including Mph) in the control on growth of metastases is well known [7]. The antimetastatic effect of Lp-Acr is probably connected with its more marked activating action on pulmonary (alveolar) Mph, a conclusion based on our own data indicating higher activity of Lp-Acr than that of the three antibiotics with respect to induction of the tumoricidal properties of Mph.

Lp-Acr thus possesses activity against Ehrlich's ascites carcinoma comparable with that of free Acr (if used in high doses), it has a more marked antimetastatic action, is free from toxicity (in the doses used), and has higher activating capacity toward Mph than the original antibiotic. The results of this investigation indicate the desirability of creating liposomal forms of drugs which have the properties of both cytostatics and Mph activators.

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