Evaluation of Antitumor Activity of Rubomycin Deposited in Absorbable Polymeric Microparticles

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 3, pp. 333-336, March, 2008
Original article submitted July 2, 2007

An experimental dosage form of rubomycin is developed: the drug is incorporated in absorbable polymeric (polyhydroxybutyrate) matrix in the form of microparticles. Antitumor efficiency of this rubomycin dosage form was studied in laboratory mice with transplanted Ehrlich ascitic carcinoma. Rubomycin deposited in polymeric microparticles exhibited pronounced antitumor activity, inhibited the proliferative activity of Ehrlich ascitic carcinoma, and improved survival of mice with tumors. This dosage form of the drug can be used for local injections.

Key Words: *microencapsulation; rubomycin; absorbable polymers; polyhydroxybutyrate; Ehrlich's ascitic carcinoma*

Rubomycin is an effective antitumor drug widely used in chemotherapy [7,8]. Contact of rubomycin with tissues causes necrosis, and hence, it is administered only intravenously; high toxicity of rubomycin can cause serious side effects [12]. In order to reduce the total toxicity and prolong the *in vivo* circulation of rubomycin, erythrocytes were proposed as the carriers [1] and conjugates with high molecular-weight carrier compounds, such as polyethylene glycol, *N*-(2-hydroxypropyl)metacrylamide copolymer, were studied [10].

Rubomycin is a high molecular-weight compound easily soluble in water and many solvents which impedes the creation of stable systems for prolonged drug delivery; the development of these systems is now the most promising and rapidly developing trend in pharmacology [10].

An experimental form of rubomycin deposited in thermosensitive micelles from isopropylacrylamide and polylactide/glycolide copolymer was described, which proved to be highly toxic towards 4T1 mouse mammary carcinoma cells [13]. The pH-sensitive copolymer micelles from polylactide and L-histidine with deposited rubomycin were developed, which are characterized by pronounced *in vitro* and *in vivo* cytotoxic effects towards rubomycin-resistant MCF tumor cells [11]; deposited rubomycin in the form of thermosensitive conjugates with polypeptides effectively inhibited the development of solid tumors [7]. The possibility of rubomycin conjugation and binding to various substances is the principal basis for its deposition in polymeric matrix.

The key to creation of long-acting dosage forms with regulated drug release is adequate material, absolutely inert and safe and characterized by a complex of certain physical mechanical and biomedical properties, including degradation in biological media. Among the materials used and studied with this purpose at present are monocarbonic acid polymer derivatives (polylactides and polyglycolactides) and, since recent time, polyesters of microbiological origin: polyhydroxyalcanoates (PHA) [10,14]. These are linear polyesters, characterized by biodegradation and biocompatibility, with good

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0007-4888/08/1453-0358

prospects for the use in medicine, pharmacology, and other spheres [2]. Complex studies of these polymers, carried out at Institute of Biophysics, demonstrated they were fit to be used as the functioning cell matrices for restoration of damaged tissues and for drug deposition [2,5,6].

We studied antitumor efficiency of rubomycin, deposited in PHA polymeric matrix in the form of microparticles (RPM), in experimental animals.

MATERIALS AND METHODS

A β-hydroxybutyric acid polymer (polyhydroxybutyrate, PHB), the most widely used and best studied representative of the PHA family, was studied as a polymeric carrier. The characteristics of highly purified polymer specimens synthesized according to the technology developed at Institute of Biophysics, were as follows: molecular weight 150 kDa, 72% crystallinity, and melting temperature 168°C [2]. The material is registered as Bioplastotan [4]. Experiments were carried out with daunorubicin (rubomycin hydrochloride; Bryntsalov-A Company).

The microspheres were obtained by solvent evaporation from emulsion (PHB solution in dichloromethane with polyvinyl alcohol and gelatin) [6]. The microstructure of microspheres was studied by electron microscopy (JSM T-330, Jeol); size distribution was studied using an automated particle counter (Casy TC, Scharle System GmbH).

The degree of rubomycin incorporation in the microspheres was evaluated by spectrofluorometry (Aminco Thermo Spectronic, 476 and 592 nm excitation and emission wavelengths).

Experiment was carried out on laboratory animals (BALB/c mice, 20-23 g, from Krasfarma Breeding Center; 30 animals per control and experimental groups) with transplanted Ehrlich's ascitic carcinoma (EAC), obtained from Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. Tumor cells were transplanted intraperitoneally to all animals in a dose of 3×10^6 cell/mouse in 0.2 ml saline. Animal morbidity and mortality after this EAC dose is 100%, the maximum life span after cell injection is ≤2-3 weeks. Experimental animals received 50 mg RPM (0.16 mg/animal, or 8 mg/kg) intraperitoneally simultaneously with EAC. One more group of animals received RPM in the same concentration 5 days after EAC transplantation. The animals were kept in a vivarium on standard ration [3].

The antitumor effect of RPM was evaluated by reduction of animal mortality, tumor volume reduction (in ml), and reduction of cell concentration in ascitic fluid (counted in Goryaev chanmer), as the volume of ascitic fluid and proliferative pool of tumor suspension cells are the parameters determining tumor growth rate. The percentage of necrotic cells was evaluated by trypan blue staining.

Differences were considered significant at $p<0.05$. The kinetic coefficients were calculated for evaluating the kinetics of tumor process development.

RESULTS

The microspheres prepared from 3-component polymeric emulsion were characterized by a regular spherical shape, well-developed "wrinkled" porous surface, and were heterogeneous by diameters. Microspheres with diameters of <2 μ, 2-10 μ, and >10 μ constituted 33.3±2.2, 38.0±1.9, 29.0±2.3%, respectively. The largest shells were 35 μ in diameter, their percentage not exceeding 3-5%. Microspheres about 10 μ in diameter were taken for the experiment.

Seven days after EAC transplantation, the tumor volume in control animals reached 0.62±0.08 ml; in experimental animals it was by one order of magnitude lower (0.020±0.004 ml; Table 1). Total cell

TABLE 1. Effect of RPM on the Time Course of EAC Development in Mice with Tumors ($M\pm m$)

Parameter	Day of observation			
	7		14	
	control	experiment	control	experiment
Tumor volume, ml	0.62±0.08	0.020±0.004	5.26±0.49	0
Count of tumor cells/ml	$(3.49\pm0.41)\times10^8$	$(1.25\pm0.31)\times10^8$	$(6.41\pm0.89)\times10^8$	$(1.56\pm0.23)\times10^8$
Count of necrotic cells				
per ml	$(0.68\pm0.01)\times10^7$	$(0.10\pm0.01)\times10^8$	$(0.32\pm0.09)\times10^7$	$(0.07\pm0.01)\times10^8$
% of total cell count	1.88±0.42	3.04±0.27	0.490±0.004	3.59±0.52*

Note. *$p<0.05$ compared to the control.

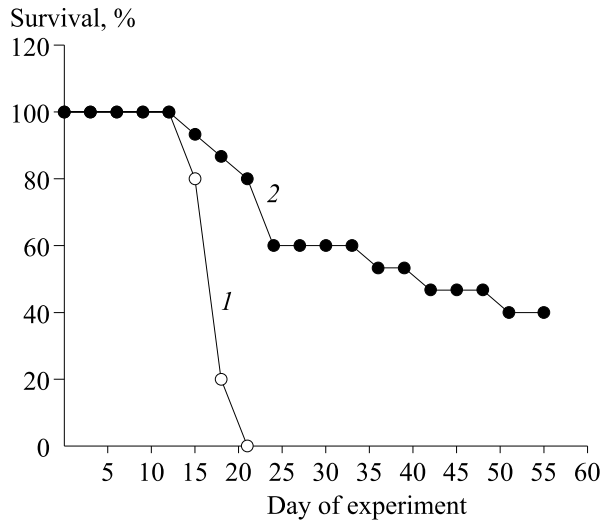

Fig. 1. Survival of mice with transplanted EAC. *1*) control group; *2*) experimental animals injected with a single dose of RPM.

counts were comparable in the control and experimental groups: $(3.49\pm0.41)\times10^8$ and $(1.25\pm0.31)\times10^8$ cells/ml ascitic fluid, respectively, while the number of necrotic tumor cells was significantly higher in animals injected with the drug, 3.04% of total cell count *vs.* 1.88% in the control. After the nest 7 days, the antitumor effect of rubomycin was even more demonstrative. Tumor volume in the controls increased by one order of magnitude and reached 5.26±0.49 ml, while experimental animals exhibited no signs of ascites. Therefore, in order to collect tumor cells, 1 ml saline was injected into the abdominal cavity of experimental animals. Total number of EAC cells increased in the controls and was 4-fold higher than in experimental animals. The number of necrotic cells in the controls decreased in comparison with day 7 postinjection, while in experimental animals this parameter increased. These results indicate RPM inhibition of proliferative activity of EAC.

Overall deaths started from day 14 in the control group, and over 8 days since then all animals died, that is, by day 21 the mortality was 100%. All dead animals had large ascitic tumors and died with symptoms characteristic of late stage of the disease (dyspnea, poor mobility, refusal from food). The mean life span of mice with tumors in the control group was 8 days. The mortality curve of mice injected with RPM simultaneously with EAC differed significantly from the control. Solitary deaths occurred starting from day 13, and by day 21, when all controls died, the survival of animals treated with RPM was 80%, the mean life span for that period being 16 days (2-fold longer than in the control). Mortality rates in experimental group decreased over 30 days, and the mortality curve looked smooth. No signs of EAC were observed in the 40% survivors on day 55.

Injection of RPM 5 days after EAC transplantation (not simultaneously with it) did not modify the development of tumor process and mortality curve.

Mathematical processing of the data on mortality of mice with tumors by the method of least squares provided kinetic parameters, characterizing antitumor activity of RPM. The kinetic coefficients of mortality intensity (c) in control mice with tumors were 1.96 *vs.* 0.23 in the experimental group. The kinetic coefficient c (kinetic curve coincidence coefficient, called tumor process inhibition coefficient) directly shows, how many times slower (in comparison with the control) the tumor process develops in animals treated with antitumor drugs, and is suggested for evaluating the antitumor drug activity. The kinetic coefficient indicates, that RPM is characterized by pronounced antitumor activity and reduces mortality in animals with EAC transplanted in a lethal dose virtually by one order of magnitude in comparison with the control.

It is noteworthy that the RPM dosage form can be injected locally, without causing negative reactions, which rule out such use of free rubomycin.

The authors are grateful to Member of Russian Academy of Sciences I. I. Gitel'zon and Member of Russian Academy of Sciences and Russian Academy of Medical Sciences A. I. Vorobyov for attention to the study and valuable recommendations in discussion of the results.

The study was supported by the Ministry of Education and Science of the Russian Federation (grant No. R1MO002) and the US Civil Research and Development Foundation (grant No. RUX0-002-KR-06), Programs of the Board of Russian Academy of Sciences "Basic Sciences for Medicine" (project No. 12.5) and Program of Interdisciplinary Integration Projects of Siberian Division of Russian Academy of Sciences (projects Nos. 14 and 54).

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