Increase in Sarcolysin Antitumor Activity by Transforming It into a Lipid Derivative and Incorporation in the Membrane of Liposomes Containing a Carbohydrate Vector

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Lipid derivative of sarcolysin obtained by condensation of sarcolysin with dioleoylglycerol is well retained in the liposome membrane due to high hydrophobic activity exhibits a higher cytotoxic activity than sarcolysin *in vitro* (CaOv cells) and a much higher antitumor activity *in vivo* (P388 leukemia). Further increase in the efficacy of the lipid derivative *in vivo* is attained by incorporating a carbohydrate vector in the liposomal membrane.

Key Words: *liposomes; sarcolysin; lipid derivative; purposeful delivery; P388 leukemia*

The tissue distribution, pharmacokinetics, and membrane transport of antitumor drugs can be modified by liposomes. This method is not widely applied in clinical practice [5,9]. Water-soluble compounds have been used for this purpose; they are encapsulated into liposomes, which requires special methods of preparation and hampers the use of such agents. Moreover, a considerable portion of the drug may be released in the extracellular space during fusion of the liposome with the cell [5], thus abating the therapeutic effect.

Introduction of antibodies to specific tumor antigens in liposomal membrane through a lipid anchor seems a promising way to improve the efficacy of targeted delivery of liposomes to tumor cells [8]. The obvious shortcomings of this method are the need in antibodies, which are difficult and expensive to obtain, and a high probability of immune response to the antibodies.

Attempts to overcome these difficulties by transforming the cytostatics in lipid derivatives insoluble in aqueous media but capable of integrating in the bilayer liposomal membrane have been made. When the liposomes fuse with cells, the cytostatic lipid derivative incorporates in the plasma membrane, enters the cell, and after cleavage by enzymes (esterases or phosphatases — according to the type of the derivative) releases the initial substance [3,4].

Recent studies showed that tumor cells express glycoprotein molecules on their surface. These glycoproteins were named lectins; they specifically bind to certain mono- or oligosaccharides and are few or absent on the surface of normal cells [6]. Thus, it can be suggested that modification of liposomal membrane with carbohydrate vectors can increase their tropism to tumor.

The aim of this study was to compare the cytotoxic and antitumor activities of liposomes containing sarcolysin lipid derivative (SLD) and to assess the possibility of increasing these activities with two simple vectors carrying α-L-fucose (α-L-Fuc) and N-

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TABLE 1. Cytotoxic Activity of Sarcolysin-Containing Compounds for CaOv Cell Culture

Compound	Sarcolysin concentration, M	Inhibition of ^{3}H-thymidine incorporation in CaOv cells, %
Sarcolysin	5×10^{-5}	80±12
	5×10^{-6}	26±4
	5×10^{-7}	5±1
Liposomes (empty)*	5×10^{-5}	13±2
	5×10^{-6}	6±1
	5×10^{-7}	0
Liposomes with vector*	5×10^{-5}	14±2
	5×10^{-6}	7±1
	5×10^{-7}	3±1
Liposomes with SLD	5×10^{-5}	90±14
	5×10^{-6}	71±10
	5×10^{-7}	24±4
Liposomes with SLD and vector	5×10^{-5}	91±14
	5×10^{-6}	66±9
	5×10^{-7}	12±2

Note. *The preparations were added to culture medium in the same concentration (by total lipids) as the SLD-containing liposomes.

acetyl-β-D-galactosamine residues as ligands for tumor lectins.

MATERIALS AND METHODS

SLD is a sarcolysin esterified with 1,2-dioleoylglycerol residue. Lipophilic carbohydrate vectors are the conjugates of saccharides (15 mol/dl) and phosphatidylethanolamine (15 mol/dl) with polyacrylamide matrix (about 40 kD) [7] with α-L-fucose (vector 1) and N-acetyl-β-D-galactosamine (vector 2).

Liposomes consisting of egg phosphatidylcholine, phosphatidylglycerol, cholesterol, and α-tocopherol in molar ratio of 97:8:37:1, to which 10 mol/dl of SLD was added, were prepared by ultrasonic treatment, followed by adding about 0.014 mol/dl the vector. The concentration of SLD in the final suspension was 0.44 mM.

The cytotoxic activity of compounds was routinely tested in human ovarian carcinoma cell culture (CaOv strain) [1].

Antitumor activity was assessed from the prolongation of the mean life-span of BDF1 mice with experimental P388 leukemia after intraperitoneal injection of the tested agents in doses of 7.0, 3.5, and 0.7 mg/kg 48 h after transplantation of leukemia cells and repeatedly after 72 h [2].

RESULTS

Table 1 shows that incubation of CaOv cells in medium with 5×10^{-5} M sarcolysin resulted in 80% inhibition of ^{3}H-thymidine incorporation. In lower concentrations sarcolysin was inactive.

Free liposomes and liposomes modified with α-L-Fuc-containing vector were similarly inactive. Liposomes containing SLD showed relevant activity at sarcolysin concentrations lower by an order of magnitude.

SLD-containing liposomes with sarcolysin concentrations of 5×10^{-6} M inhibited the incorporation of ^{3}H-thymidine by CaOv cells by 71%, whereas the same concentration of free sarcolysin was ineffective.

It is noteworthy that further modification of liposomes with α-L-Fuc-containing vector did not alter their cytotoxic effect.

An increase in cytotoxic activity of SLD in liposomes was confirmed *in vivo*. Table 2 shows that sarcolysin in a dose of 7.0 mg/kg injected twice at a 72-h interval prolonged the mean life-span of mice with experimental leukemia by 113%. Free liposomes were ineffective. On the other hand, liposomes containing SLD in sarcolysin equimolar concentration were characterized by more pronounced antitumor activity.

SLD in liposomes additionally modified with carbohydrate vectors still more intensely inhibited the development of experimental leukemia. The mean life-

TABLE 2. Effect of SLD-Containing Liposomes on the Life-Span of BDF1 Mice with Experimental P388 Leukemia

Drug	Treatment protocol, ml/72h×2	Sarcolysin dose, mg/kg	Prolongation of life-span, %
Sarcolysin	1.0	7.0	113
	0.5	3.5	40
	0.1	0.7	20
Liposomes (empty)	1.0	—	3
	0.5	—	23
	0.1	—	10
Liposomes with SLD	1.0	7.0	163
	0.5	3.5	100
	0.1	0.7	40
Liposomes with SLD and vector 1	1.0	7.0	213
	0.5	3.5	133
	0.1	0.7	47
Liposomes with SLD and vector 2	1.0	7.0	243
	0.5	3.5	147
	0.1	0.7	30

span of animals injected SLD in liposomes additionally modified with N-acetyl-β-D-galactosamine-containing vector was 243% longer than in the control.

Our results indicate that SLD incorporated in liposomal membrane exhibits a much higher antitumor activity than that of intact sarcolysin. Further modification of SLD-containing liposomes with carbohydrate vectors increases their activity.

Fucose and galactosamine derivatives were used for liposome modification as agents presumably affine for lectins of a wide spectrum of tumors. We used these carbohydrates to modify liposomal membranes but did not clear out the specificity of tumor cell binding to membranes. At the same time, the specificity of the interaction appears to be as real as the relationship between the effect and the vector affinity to tumor cell plasma membrane.

At present, we search for the optimal vector to attain the potentially possible tropism of liposomes to tumor cells and study the antitumor activity of new agents.

REFERENCES

1. N. A. Rzaeva, F. I. Abdullaev, T. P. Ivanova, and Ya. V. Dobrynin, *Eksper. Onkol.*, 7, No. 6, 67-69 (1985).
2. Z. P. Sof'ina *et al.* (Eds.), *Experimental Assessment of Antitumor Drugs in the USSR and the USA* [in Russian], Moscow (1980).
3. L. Elkihel, M. Gelin, and Y. Letourneux, *Arzneimittelforschung*, **45**, No. 1-2, 190-194 (1995).
4. K. Hashimoto, J. E. Loader, and S. C. Kinsky, *Biochim. Biophys. Acta*, **816**, No. 2, 169-178 (1986).
5. D. D. Lasic and D. Papahadjopoulos, *Science*, **267**, 1275-1276 (1995).
6. O. Molema and D. C. F. Meijer, *Adv. Drug Deliv. Rev.*, **14**, 25-48 (1994).
7. M. Monsigny, A.-C. Roche, P. Midoux, and R. Mayer, *Ibid.*, pp. 1-24.
8. U. K. Nassander, P. A. Steerenberg, H. Poppe, *et al.*, *Cancer Res.*, **52**, No. 2, 646-653 (1992).
9. R. Perez-Soler, *Cancer Treat. Rev.*, **16**, 67-82 (1989).