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PRODUCTION OF LIPOGLYCOPROTEIN MICELLES WITH ARGLABINE ANTITUMOR PREPARATION

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The possible production of a new medicinal form of the antitumor preparation arglabine was studied by including it in lipoglycoprotein micelles isolated from wheat grains.

Key words: lipoglycoprotein micelles, arglabine, sesquiterpene γ-lactone, medicinal form.

Targeted delivery of medicines has acquired a special significance because scientists have succeeded in unraveling the mechanisms of onset and progression of many diseases and in producing a wide spectrum of biologically active compounds that can affect many pathological processes on the molecular level. As a rule, preparations are more or less evenly distributed throughout an organism and do not selectively target the diseased site. This causes indesired side effects on healthy organs and tissues because of heightened activity. Therefore, targeted delivery of medicines is being investigated in various countries. Several approaches to developing such systems are based on these results.

Binding of medicinal molecules and other molecules for which the diseased organ or its cells are considered the natural targets is the most common method. Such molecules (vectors) can be compounds as varied as hormones, lectins, proteins, enzymes, glycolipids, glycoproteins, etc.

A promising medicinal substance is the new antitumor preparation arglabine, which is based on a sesquiterpene γ-lactone isolated from smooth wormwood (*Artemisia glabella* Kar. et Kir.). Amination and hydrochlorination of it produces a water-soluble form.

Natural amphiphilic lipids can be used to prepare microcapsules. They satisfy the requirements needed for ideal carriers of medicines because natural amphiphilic lipids are biodegradable and do not accumulate in the organism. They are obtained from natural sources such as soy, egg yolk, and brain tissues of animals [1-3]. The richest sources of phospholipids (PL) are egg yolk (20% dry weight) and mammal brain tissue (30.9%). Soybeans and cotton and sunflower seeds contain from 0.5 to 2.5% [4].

The biological properties of phospholipids depend on the physicochemical properties of the preparation. The energy content comes from the fatty-acid (FA) composition of the oily phase; the isotonicity, the composition of the aqueous medium; the apyrogenicity and toxicity, the chemical structure of the emulsion components. We prepared phospholipid micelles using wheat grain (Saratov-29) and phosphatidylinositol, which according to the literature [5] contains the following principal acids (%): 16:0, 21.81; 18:1, 17.70; and 18:2, 29.97.

The length of the hydrocarbon part of the molecule affects not only the stability of the aggregates formed but also their properties [6]. It was demonstrated that PL with FAs containing less than eight C atoms form micelles (aggregation number ~500). Increasing the length of the FA chains of the PL, judging from the aggregation number (3000-6000) forms dispersions of liposomes.

The surface tension of the membrane lipid bilayer is minimal at neutral pH values and maximal at low ones. The resistance of the lipid surface is highest. Therefore, the stability of this surface is also high. A deviation of the pH from the optimal value increases the electrostatic repulsion of the lipids and decreases the packing density of the monolayer. Thus, the

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effect of pH and ionic strength of the medium on the properties of the interphase surface is obvious. Therefore, fatty emusions should have a neutral pH not only because of considerations about the blood pH but also to ensure the required concentration of preparation.

It was demonstrated that empty micelles and micelles with arglabine are stable at pH 7.4 for extended periods (depending on storage temperature). Storage of micelles in buffers with pH 1.68, 4.01, 6.86, and 9.18 causes rapid destruction in acidic medium (3 d at 20°C). They are sufficiently stable in alkaline medium (21 d at 20°C).

Thermal rearrangements in lipid aggregates occur if the temperature is varied. Defects in the lipid packing precedes its destruction. The probability of forming such defects is related to the temperature rise. Therefore, it is natural to expect that the stability of the lipid structure will decrease if the temperature is raised above a certain point. In most instances, lipids of biological origin, which are used as emulsifiers, are a complicated multicomponent mixture of PLs, glycolipids, and sterols. Therefore, phase transitions occur in such systems over a wide temperature range. For example, phosphatidylcholines of human erythrocytes have a transition range from -30 to 10°C; sphingomyelins of human erythrocytes, 20-37°C; total brain PLs, 25-65°C, lipoglycoproteins micelles produced by us, -10 to 55°C [5].

It was noted that empty micelles and micelles with arglabine at -10°C are stable up to one year and longer. At 20°C, the stability depends on the loading of the preparation and reached 90 d for 1.25 mg/mL arglabine and 5 d for 50 mg/mL. The loaded micellar complex withstands heating to 35-45°C depending on the amount of bound medicine.

A new medicinal form of the antitumor preparation arglabine is produced by including it in lipoglycoprotein micelles. The complex has a high capacity relative to the preparation and is sufficiently stable over a wide temperature range from -10 to 35°C and pH values of 4.01, 7.4, and 9.18.

EXPERIMENTAL

The yield of micelles loaded with arglabine preparation was monitored by a system consisting of an LKB 2238 UVICORD S II photometer (Sweden) with $\lambda = 254$ nm, an FCC 60 Fraction Collector, and a 2210 2-Channel Recorder. UV spectra were recorded on an SF-26 instrument. Eluent was *tris*-chloride buffer (pH 7.4). Micelles were separated by filtration through a 200-nm pore diameter membrane filter (Sartorius AG, Germany).

Preparation of Lipoglycoprotein Micelles. Wheat grain (Saratov-29) was used to prepare PL micelles. The germ was removed. The material was finely ground in 0.05 M *tris*-chloride buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant liquid after centrifugation was chromatographed on Sepharose 2B to remove the micelle fraction. It was shown [5] that the micelles are protein—glutamatedehydrogenase, which is bound through the C-terminus to oligosaccharide (glycan) bonded to phosphatidylinositol located in the lipid bilayer of the cell membrane.

Study of Lipid Composition. Spherosomes that were purified by gel-chromatography on Sepharose 2B and isopycnic ultracentrifugation were extracted according to Folch. The PL extract was separated on silica-gel plates using hexane:diethylether:acetic acid (73:25:2). Lipids of the spherosomes were not extracted by petroleum ether or ethanol. Therefore, the spherosomes consist only of PLs. According to chromatography, the spherosomes include only one lipid class, PL. The specific PL was determined by TLC on silica gel using two solvent systems: $CHCl_3$:CH_3OH:NH_3 (7 N) (35:60:5) and $CHCl_3$:CH_3OH:CH_3CO_2H:H_2O (50:25:8:4).

Chromatographs were developed in iodine vapor or by spraying with ammonia silver nitrate. It was found that the spherosomes contain only one PL. According to specific coloration of ammonia silver nitrate and the chromatographic mobility, it is phosphatidylinositol.

Physicochemical characterization was performed to prove that the functional group of the spherosome PL was inosite. The studied PL was hydrolyzed for 24 h at 125°C in a thermostat.

The contents of the ampul were filtered after hydrolysis into a separatory funnel. An equal volume of $CHCl_3$ was added to remove FAs and triacylglycerides, which transferred into the $CHCl_3$ on shaking. The functional group of the PL was separated. Traces of HCl and water were removed by evaporating to dryness in a rotary evaporator. The resulting crystalline substance contained traces of phosphoric acid, which was removed by washing with absolute ethanol on a glass filter and drying in a vacuum desiccator over granulated NaOH.

Thus, a water-soluble white crystalline powder with mp 210°C was obtained. A commercial sample of inosite that we used as a control behaved analogously with respect to solvents.

Production of Lipoglycoprotein Micelles Loaded with Arglabine. A column of Sepharose 2B gel was equilibrated with *tris*-chloride buffer. A certain volume of aqueous arglabine preparation of given concentration, 96% ethanol, micelle solution, and ethanol again were placed on it. The mixture was eluted with *tris*-chloride buffer. The yields of loaded micelles and unbound arglabine were monitored using the change of optical absorbance of the column effluent.

Destruction of Loaded Micelles. Micelles loaded with arglabine (1 mL) were separated from the solution by filtration and treated with ethanol (10 mL). The optical absorbance of the resulting alcohol solution was measured at $\lambda = 204$ nm.

Arglabine Determination. A series of arglabine-preparation solutions in 96% ethanol of concentrations 5.0, 10.0, 20.0, 40.0, and 60.0 μg/mL was prepared. Their spectra in the range 200-400 nm relative to solvent were recorded. Then, a calibration curve at $\lambda = 204$ nm was constructed, from which the preparation concentration was determined. The change of optical absorbance obeyed the linear equation $y = 0.0125x + 0.1504$.

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