DEVELOPMENT OF THE TECHNOLOGY OF A LIPOSOMAL FORM OF EYE DROPS BASED ON A PEPTIDE COMPLEX

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Abstract: The aim of this work is to develop the stage of inclusion of the active pharmaceutical ingredient in liposomes, to study the effect of liposome composition on the degree of encapsulation and particle size in creating a liposomal eye drops based on a peptide complex used as a drug for ophthalmic diseases. Different concentrations of lipid membrane 80 mg/mL, 90 mg/mL, 100 mg/mL were studied. The influence of particle size on the degree of encapsulation of the peptide complex from 300 nm to 80 nm was studied. The highest degree of encapsulation of the peptide complex was determined for a liposomal sample with a lipid concentration of 100 mg/mL and a particle size of 120-140 nm or less.

Keywords: eye drops, peptide complex, liposomes, stage of inclusion, degree of encapsulation, particle size

Serious medical and social problems are eye burns, diseases, and injuries of the cornea, which have a high share in the etiology of poor vision and blindness. Modern methods of treatment of burn eye disease can increase the frequency of successful conservative therapy of burn eye injury and reduce the percentage of complications.

Comprehensive treatment of diseases of the cornea with a violation of the integrity of its surface, trauma, and burns of the eye requires accelerating its regeneration and improving metabolic processes. Of great importance in the treatment of corneal lesions are drugs of animal and plant origin that stimulate regeneration and contain biologically active substances, vitamins, enzymes, trace elements, etc. (1, 2).

The small range on the pharmaceutical market of Europe of drugs of animal origin, made, in particular, on the basis of peptide complexes contained in the skin of pigs, necessitates the development and creation of such drugs.

Ophthalmic diseases are quite difficult to treat; ophthalmic forms of the drug must be safe, hypoallergenic, and sterile. Topical forms account for 90% of all registered drugs. Tear fluid renewal, nasolacrimal drainage, corneal epithelium, and blood-ophthalmic barrier reduce the local bioavailability of the drug and their residence time on the surface of the eye in topical application. Only 5-10% of the drug penetrates the corneal barrier. The creation of drugs based on nanoparticles is one of the promising areas of modern nanobiotechnology.

A number of nanostructures used as drug carriers are well known: polymeric nanoparticles; liposomes; nanodispersions of oil and water; cyclodextrins; metal nanoparticles and a number of others. They have many advantages: prolong the residence time of the drug on the surface of the cornea, prolong its release and increase bioavailability (3, 4).

To avoid problems of poor bioavailability, as well as side effects, various drug delivery systems for the treatment of ophthalmic diseases, including nanomaterials based on polymers and lipids, have been studied. The most studied nanocarriers in ophthalmic diseases are liposomes, which have the advantages of biocompatibility and biodegradation (5, 6).

Some liposomal drugs are already used in clinical trials for eye diseases. Various delivery routes and formulations have been developed to optimize the delivery of liposomal drugs to the anterior or posterior segment of the eye by altering the surface charge or lipid composition.

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In this post, we will focus on one of the major critical stages in the production of a liposome-based drug. One of the most important steps in a liposome-based drug is the incorporation of the active pharmaceutical ingredient into the liposomes. Today there are several methods of including the active substance in liposomes: in the internal volume of nanoparticles; in the hydrophobic region of the lipid biolayer; by adsorption on the surface of the drug; due to chemical binding with the components of the biolayer (7, 8).

The inclusion of the substance in liposomes can be realized in several ways: the formation of a lipid film with the introduction of a lipophilic drug substance, followed by hydration in an aqueous medium; hydration of the lipid film with water or a buffer solution containing the active pharmaceutical substance; by the method of a chemical gradient of ions (9, 10).

The effectiveness of the inclusion – one of the most important parameters of liposomal drugs – is calculated as the ratio of the mass of the substance associated with liposomes to the total mass of the substance taken to prepare a liposomal dispersion. The efficiency of inclusion depends on the partition coefficient and the proportion of the volume of dispersion occupied by liposomes. The higher the partition coefficient, the more substance will be in the membrane, i.e., in the liposomes (11).

Therefore, the effectiveness of the inclusion of lipophilic and hydrophobic substances is mainly determined by the concentration of lipids and the solubility of a particular lipophilic substance in this phase. Not included hydrophobic substance due to insolubility in water forms a separate phase, which can usually be separated by filtration (12, 13).

Liposomes can include substances of different classes, with low molecular weight water-soluble drugs, are included mainly in the inner aqueous phase of liposomes, and high molecular weight lipophilic substances are sorbed on their outer surfaces, mainly due to the formation of hydrogen bonds with polar groups of lipids.

Properties of liposomes that give them an advantage over other drug carriers:
First, it is related to the natural membranes of cells in chemical composition. It is known that lipids are part of the membranes and occupy from 20 to 80 percent of their mass. Therefore, with the correct selection of liposome components, their introduction into the body does not cause adverse reactions (14, 15).

Secondly, an important property of liposomes is universality. Due to the semi-synthetic nature, you can widely vary their size, characteristics, surface composition. This allows “liposomes” to carry a wide range of pharmacologically active substances: anticancer and antimicrobial drugs, hormones, enzymes, vaccines, as well as additional energy sources for the cell, genetic material, active pharmaceutical ingredients of biological origin.

Third, liposomes are relatively easily destroyed in the body, releasing delivered substances, but in the passage, liposomes lack the properties of antigen, reliably protect the encapsulated active pharmaceutical ingredient from contact with the immune system, and, therefore, do not cause protective and allergic reactions.

An important role is played by the nature of the interaction of liposomes with cells: from simple adsorption to fusion with the cell membrane (16, 17).

All of the above properties suggest that the use of liposomes as a carrier of the deproteinized dermal layer of pigskin for wound healing drugs will provide the best bioavailability (18).

The aim of the work is to develop the stage of inclusion of the active pharmaceutical ingredient in liposomes, to study the effect of liposome composition on the degree of encapsulation and particle size in creating liposomal eye drops based on a peptide complex used as a drug for ophthalmic diseases.

**MATERIALS AND METHODS**

For the manufacture of liposomes, used lipids were manufactured by Lipoid, Germany. Crystalline glycine, edetate disodium, sodium chloride, sodium hydroxide, hydrochloric acid were manufactured by Sigma-Aldrich, USA.

Preparation of model samples of liposome suspension
Water for injection was loaded into the prepared reactor and cooled to 20-25°C. Stirring was switched on and the appropriate amount of Lipoid C100 substance was loaded. Stir for 2 hours until a homogeneous solution is formed.

The reactor was connected to a Microfluidizer M-110P (Microfluidics, USA), dispersed to set particle size (pressure 20000 PSI).

Inclusion of peptide complex in liposomes
With stirring, the resulting solution was cooled to a temperature of 8-11°C. The calculated amount of pre-thawed concentrate of the deproteinized dermal layer of pigskin was added. The solution was stirred for 30 min. After stirring, the microfluidizer was turned on and dispersed to the set particle size (pressure 20000 PSI). To the resulting suspension,
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A sample was taken to measure the pH of the resulting solution using a pH meter Seven Compact (Mettler Toledo, USA), if necessary, adjust the pH of the solution with solutions of hydrochloric acid and sodium hydroxide to 5.5 ± 0.3.

Morphological examination of liposomes

For morphological examination of liposomes, the samples were centrifuged using centrifuge tubes with a selective filter (Nano Sept 10k Omega, Pall) at 10000 rpm for 15 min. The liposome samples separated on the filters were placed on tables and allowed to dry in a desiccator.

Subsequently, the samples were covered with a layer of conductive material (a coating of 25 nm Au/Pd alloy was used). Morphology was observed using a Transmission Electron Microscope (Hitachi S-4800, Hitachi Ltd., Japan).

Research by the method of dynamic light scattering

Liposome suspension studies were performed on a Zetasizer Nano ZS (Malvern Instruments, UK). The samples were filtered through a 0.2 μm membrane filter and diluted 1: 40 in 0.02 μm filtered PBS.

The sample was added to a disposable polystyrene cuvette 10 x 10 x 45 mm. The cuvette was placed in a Zetasizer Nano. Measurements were performed.

Results and discussion

For the development of the drug, we chose phosphatidylcholine soybeans (Lipoid C100). The concentration of phosphatidylcholine from soybeans (Lipoid C100) was determined empirically to ensure the inclusion of peptides of the dermal layer of pigskin in liposomal form.

During the pharmaceutical development of the drug, model samples with different concentrations of phosphatidylcholine from soybeans (Lipoid C100) were prepared (Table 1). Inclusions were performed under the same conditions.

To determine the percentage of inclusion of peptides of the deproteinized dermal layer of pigskin in the liposome, the obtained samples were applied transmission electron microscopy, the results are presented in Figure 1.

As can be seen from Figure 1 method of transmission electron microscopy did not make it possible to distinguish the included peptides of the dermal layer of pigskin in liposomal form. Also, this method does not distinguish between placebo liposomes (without the addition and inclusion of peptides of the dermal layer of pigskin) from liposomes with included peptides.

For further work to determine the percentage of inclusion of peptides of the deproteinized dermal layer of pigskin in the liposome the method of dynamic light scattering was used. The results are presented in Figure 2.

As can be seen from Figure 2 method of dynamic light scattering makes it possible to distinguish the included peptides of the dermal layer of pigskin in the liposome from placebo, but this method does not allow to determine the percentage of inclusion of peptides in the liposomal form.

Table 1. Model samples for the selection of the concentration of phosphatidylcholine from soybeans (Lipoid C100).

| Component                                      | Sample 0 (placebo) | Sample 1 | Sample 2 | Sample 3 |
|------------------------------------------------|--------------------|----------|----------|----------|
| Deproteinized dermal layer of pigskin (in terms of peptides) | 0 mg/mL            | 1.5 mg/mL| 1.5 mg/mL| 1.5 mg/mL|
| Phosphatidylcholine (Lipoid C100)               | 100 mg/mL          | 80 mg/mL | 90 mg/mL | 100 mg/mL|
| Diluted hydrochloric acid                       | up to pH 5.0-6.0   | up to pH 5.0-6.0 | up to pH 5.0-6.0 | up to pH 5.0-6.0 |
| Sodium hydroxide                                | up to pH 5.0-6.0   | up to pH 5.0-6.0 | up to pH 5.0-6.0 | up to pH 5.0-6.0 |
| Water for injections                             | up to 1 mL         | up to 1 mL | up to 1 mL | up to 1 mL |
Figure 1. Transmission electron microscopy images of liposomes of Sample 0 (placebo) (1), liposomes of Sample 1 (2), liposomes of Sample 2 (3), liposomes of Sample 3 (4).

Figure 2. The results of the study by the method of dynamic light scattering of Sample 0 (placebo), Sample 1, Sample 2, Sample 3.
To determine the percentage of inclusion of peptides in the liposome, the obtained model samples 0-3 were pre-centrifuged using centrifuge tubes with a selective filter (Nano Sept 10k Omega, Pall).

The content of peptides in the dermal layer of pigskin was analyzed in the supernatant (free of liposomes with included peptides) and calculated as the percentage of included peptides (it was assumed that peptides not included in the liposome remain in the supernatant during centrifugation of the sample). The results obtained are presented in Table 2.

As can be seen from Table 2 in sample No. 3, where the concentration of phosphatidylcholine from soybeans is 100 mg/mL, there is a 100% inclusion of peptides of the dermal layer of pigskin in the liposome.

Therefore, the amount of phosphatidylcholine from soybeans (Lipoid C100) in the developed drug is 100 mg/mL.

The particle size of the emulsion sufficient for the transition to the next stage was established empirically: for a solution of phosphatidylcholine soybeans of different sizes was included protein deproteinized dermal layer of pigskin at an operating pressure of 20000 PSI for 20 min, controlled the percentage of protein inclusion. The results are shown in Table 3.

As can be seen from Table 3, the full inclusion of the protein of the deproteinized dermal layer of pigskin in 1 liter of liposome solution at an operating pressure of a dispersant of 20000 PSI for 20 min occurs in solutions of liposomes with a size of 120-140 nm and less.

Since to obtain smaller liposomes it is necessary to disperse for a longer time, the most optimal from a technological point of view is a solution with a liposome size of 120-140 nm to obtain a liposomal drug in the form of eye drops.

CONCLUSION

The stage of inclusion of the active pharmaceutical ingredient in liposomes, the study of the influence of the composition of liposomes on the degree of encapsulation and particle size in creating a liposomal form of eye drops based on a peptide complex have been investigated. Different concentrations of lipid membrane 80 mg/mL, 90 mg/mL, 100 mg/mL were studied. The influence of particle size on the degree of encapsulation of the peptide complex from 300 nm to 80 nm was studied. The highest degree of encapsulation of the peptide complex was determined for a liposomal sample with a lipid concentration of 100 mg/mL and a particle size of 120-140 nm or less.

Conflict of interest

The authors declare no conflict of interest.

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