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PROCEDURES FOR PREPARING LIPOSOMAL CARRIERS WITH ANTIOXIDANT ACTIVITY

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The paper presents procedures for preparing liposomal drugs and highlights the advantages and disadvantages of the existing methods. It considers the problems associated with technology of hydrophobic antioxidants (the low solubility and chemical stability of these pharmacological substances) and the advantages of liposomes for their delivery. The most promising technology for obtaining liposomes with drug substances having antioxidant properties has been ascertained to be a thin-film hydration method, followed by vocalization.

Key words: *antioxidants, liposomes, phospholipids, directed transport, thin-film hydration method, sonication.*

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INTRODUCTION

Over the past few decades all over the world,researchershave been actively studyinghow to prepare innovative drugswith directional transport ortargeted drug delivery systems. Such systems would allow to control drug distribution in tissues, adsorption, metabolism and excretion of the drug from the patient, and to improve their bioavailability and biocompatibility [1–5].

Carrierson the basis of phospholipids (liposomes) have been of particular interest in the development of targeted drug delivery systems. Due to a number of their advantages, via liposomes can be transported very wide range of drugs – from traditional medicinal substances to genetic structures. Many drugs encapsulated in liposomes have already found application in medicine [6 – 8].

Drug transport plays an important role in pharmacology of new antioxidant agents [9]. The antioxidant agents often have low solubility, poor chemical stability and, in this regard, dissolve rapidly in the gastrointestinal tract [10]. In order to overcome these problems, antioxidant agents are used in a liposome form.

The aim of this work is aninformation-analytical study on methods ofpreparing liposomal drugs with antioxidant properties.

The literature describes various methods for the preparation of liposomal particles. Some of the most popular: convection, sonic methods, high pressure method, the dissolution and removal of the detergent method and the reverse phase evaporation method [11,12].

Of the antioxidants, which are proposed in the literature for liposomal delivery systems, the most used are flavonoids and vitamins (such as α-tocopherol and β-carotene).Liposomes with dihydroquercetin, α-tocopheroland emoxipin significantly reduce the rate of lipid peroxidation and increase plasma antioxidant activity

of the rats' blood, which attests to their ability to provide an antioxidant effect at the cellular level.

It has been found that liposomes containing quercetin reduce lipid peroxidation, whereas liposomes containing dibornol and gallic acid, as well as the empty liposomes increase the rate of lipid peroxidation while increasing antioxidant activity in blood plasma of rats [13].

The most commonly used method to incorporate antioxidants in the in liposomal membrane is thethin-film hydration method. As results, multilamellarvesicles are formed, and then small unilamellar vesicles are retrieved by passing the slurry through extruder filters [14].

According to L.A. Zabodalova et al. [15], the preparation of liposomes using soybean lecithin and β-carotene as an antioxidant may be done by dehydration/rehydration and thermal methods.

According to the classical procedure of dehydration/rehydration all operations should be carried out in an atmosphere of nitrogen or argon to protect lipids from oxidation by atmospheric oxygen. In these papers, in order to protect the lipid, in the mixture before evaporation,authors added natural antioxidant – α-tocopherol (vitamin E) in an amount of 0.01% by weight of lecithin.

Lecithin and β-carotene were mixed in a weight ratio of 1: 0.005, were dissolved in hexane and vitamin E was added in an amount of 0.01% by weight of lecithin. The solvent was evaporated on a rotary evaporator at a bath temperature – 45–50°C. To the residue after evaporation, a mixture of water-ethanol (1: 1, V) in an amount exceeding 1.5 times the mass of lecithin was added. The flask contents were shaken until complete transfer of the residue after evaporation in a water-alcohol mixture. The resulting emulsion was left in a cool dark place. After one day, the mixture was collected, weighed and poured with distilled water so that the lipid content in the medium was 1%. The mixture was homogenized on a mechanical stirrer at 15,000 rpm for 2 minutes [15].

Electron microscopy data showed that the dehydration/rehydration method provides predominantly unilamellar liposomes. The thermal method provides multilayered liposomes. However, when analyzing the fractional-dispersed composition, 90% are coarse particles with sizes over 20 microns, the fraction with particle size of about 80 nm is obtained in a small amount [15].

For the thermal method, lecithin Epikuron 200 was filled with water so that the lipid content in the medium was 1% and left to stand for 2 hours for hydration. To the mixture they added glycerol3% (vol.), placed it in a water bath at65–70°C and stirred on a mechanical mixer at a speed of 1000 r/min for 30 min. The mixture was maintained 1 hour at theindicated temperature [15].

The literature describes a method to prepareliposomes with antioxidants – emoxipin, dihydroquercetin and α-tocopherol by extrusion through polycarbonate filters [16]. Before extrusionlipid film with desired composition was prepared,using chloroform as the solvent,on the walls of the rotary evaporator's flask. The resulting film was hydrated by internal phase solution and stirred until formation of multilamellar vesicles. To improve the hydration, the vesicles were exposed to tenfold cycle of freezing and thawing. The resulting suspension was passed through an extruder using a polycarbonate filter with a pore size of 100 nm. All of the antioxidants were added at the rate of 0.25 mmol/g of lipid.

For the preparation of liposomes with α-tocopherol and dihydroquercetin, they wereaddedin the flask at the stage of lipid film formation. Emoxipin was added as an aqueous solution to the lipid film hydration step. In the study the authors found the best membrane stabilizing activity of the resulting liposomes with emoxipin, dihydroquercetin[16].

Authors of the studies[17] considered the most suitable methods for the preparation of small unilamellar liposomes with strong antioxidant propertiesto be the extrusion method of phospholipid in aqueous suspension and the injection method in an aqueous medium a phospholipid solution in a volatile organic solvent (mostly ethanol). These methods virtually eliminate stimulation of lipid peroxidation at the stage of liposomes preparation.

The sonic methodis often used to produce liposomes. The aim of this method is to obtain a homogeneous dispersion of liposomes. Multilamellar vesicles prepared by convection method, are subjected to sonic treatment using pulsating sound waves withhigh frequency .

The disadvantages of this method are: instability of the vesicles and their propensity for fusion and thus coarsening; denaturation or inactivation of certain heat sensitive substances incorporated in liposomes, including many natural antioxidant compounds; oxidation of unsaturated bonds in the chain of phospholipids fatty acid residues; phospholipid shydrolysis to form lysophospholipids and free fatty acids [11].

In a number of studies [18,19] they mention the disadvantages of ultrasonic treatment in the preparation of liposomes that can limit its use in the preparation of liposomal drugs containing antioxidants. So, under ultrasonic treatment there is a significant lipid oxidation and hydrolysis during the insonification and a titanium contamination of the drug, capable of stimulating lipid peroxidation. This may affect both the preservation of the lipid structure forming the liposome, and the antioxidant content.

The literature also describes a reverse-phase evaporation method, well-established for the encapsulation of various compounds without loss of activity.

By this method an aqueous drug solution is quickly introduced into the organic solvent containing lipids. Simultaneously, the mixture was subjected to sonic treatment, which leads to the formation of emulsions - water droplets in an organic solvent. Next, the resulting emulsion is dried to a semi-solid gel, using a rotary evaporator. After, the gel is subjected to heavy mechanical

impact until formation of phase change from a dispersion of «water in oil» to «oil in water» (i.e., an aqueous suspension of vesicles).

During the mechanical impact water droplets form the external phase, while the other – a closed vesicles within an aqueous volume [11]. Compared with vesicles obtained by thin film hydration followed by sonic treatment, the liposomes obtained by reverse phase evaporation have a larger gripping aqueous medium volume and high encapsulation efficiency of hydrophilic drugs such as flavonoids [20]. In some cases, the method may have the following limitations:

long retention of encapsulated material in organic solvents is not always possible; mechanical processing can lead to denaturation of certain proteins or rupture of DNA chains [11].

CONCLUSION

Antioxidant agents in the form of liposomal drugs have shown high efficiency due to the synergism of action of included compounds and lipids themselves. Dihydroquercetin and emoxipinare the most effective antioxidant agents.

It was noted that the mostoften used in the preparation of liposomes – ultrasonication is not optimal for preparing liposomes containing antioxidants, as ultrasonic treatment leads to lipid oxidation and hydrolysis during the scoring, and also to drug contamination by titaniumparticles capable of stimulating oxidative processes.

The most optimal methods of preparing liposomes with antioxidants are the extrusion method and the dehydration/rehydrationmethod. These methods facilitate the obtention of unilamellar vesicles.

REFERENCES

1. Haley B.,Frenkel E. Nanoparticles for drug delivery in cancer treatment. Urologic Oncology: Seminars and Original Investigations, 2008; 26: 57–64.

2. Barenholz Y. Liposome application: problems and prospects. Curr. Opin. Colloid Interface Sci., 2001; 6: 66–77.

3. Basu M.K. Liposomal delivery of antileishmanial agents. Journal of Applied Research., 2005; 5 (1): 221–236.

4. Vasir J.K., Reddy M.K., Labhasetwar V.D. Nanosystems in drug targeting: opportunities and challenges. Current Nanoscience, 2005; 1 (1): 47–64.

5. Demina N.B. B HYPERLINK «http://elibrary.ru/keyword_items.asp?keywordid=3052917»iopharmacy – the path to creating innovative medicines. Drug development and registration, 2013; 2 (2): 8–13 (in Russian).

6. Thanos C.G., Emerich D.F. Choroid Plexus Epithelial Cell Transplants for Repair of the Brain. Cellular Transplantation, 2007; 399–416.

7. Ulrich A.S. Biophysical aspects of using liposomes as delivery vehicles. Bioscience Reports., 2002; 22 (2): 129–150.

8. Tong R., Boucher Y., Kozin S. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res., 2004; 64: 3731–6.

9. Fenske D.B., Cullis P.R. Liposomal nanomedicines. Expert Opin. Drug Deliv., 2008; 5(1): 25–44.

10. Mukhamadiarov R.A., Veremeev A.V., Martsiyash N.Y., Zinchuk V.G. The α-tocopherol influence to the lipoperoxidation process in the liposomes (in different storage temperatures) produced using ultrasonic sonification and extrusion methods. Fundamental research., 2012; 6: 566–70 (in Russian).

11. Uhumwangho M.U., Okor R.S. Current trends in the production and biomedical applications of liposomes: a review. JMBR: A Peer-review Journal of Biomedical Sciences,2005; 4 (1): 9–21.

12. Frezard F. Liposomes: from biophysics to the design of peptide vaccines. Braz. J. Biol. Res.,1999; 32 (2): 181 – 9.

13. Mukhamadiarov R.A., Bogdanov M.V., Toropova Ya.G. The study of antioxidant activity and lipid peroxidation with the administration of various liposomes with antioxidants. Modern high technologies, 2010; 12: 38–48 (in Russian).

14. Sanarova E.V., Kotova E.A., Lantsova A.V., Polozkova A.P., Oborotova N.A., Kozeev S.G., Krasnyuk I.I. Quality of liposomal phospholipids at different stages in the manufacturing process. Pharmaceutical Chemistry Journal., 2012; 46 (3): 50–3 (in Russian).

15. Zabodalova L.A., Chernjavskij V.F., Ishchenko T.N., Skvortcova N.N. Production of liposomes from soybean lecithin. Scientific Journal., NRU ITMO, 2011; 2: 75–81 (in Russian).

16. Mukhamadiarov R.A., Krivaya E.V., Kruch M.A., Plotnikov M.B. The evaluation of membrane-stabilizing effect of liposomes with different antioxidant drugs in osmotic hemolysis model. Modern problems of science and education, 2012; 4: 2827 (in Russian).

17. Zaitsev V.G. Model systems of lipid peroxidation and their application for the evaluation of the antioxidant effect of drugs.Thesis for a scientific degree. Volgograd; 2001 (in Russian).

18. Tadolini B., Franconi F. Carvedilol inhibition of lipid peroxidation. A new antioxidative mechanism. Free Radical Res., 1998; 29 (5): 377–87.

19. Plumb G.W., De Pascual-Teresa S., Buelga C.S., Cheynier V., Williamson G. Antioxidant properties of catechins and proanthocyanidins: Effect of polymerisation, galloylationand glycosylation Free Radical Res., 1998; 29 (4): 351–8.

20. Sandip B.T., Udupa N., Rao B.S.S., Devi P.U. Thermo sensitive liposomes and localized hyperthermia – an effective bimodality approach for tumor management. Indian Journal of Pharmacology, 2000; 32: 214–20.

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СПОСОБЫ ПОЛУЧЕНИЯ ЛИПОСОМАЛЬНЫХ НОСИТЕЛЕЙ С АНТИОКСИДАНТНОЙ АКТИВНОСТЬЮ

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РЕЗЮМЕ

Представлены способы получения липосомальных лекарственных препаратов, отмечены достоинства и недостатки существующих методов. Рассмотрены проблемы технологии гидрофобных антиоксидантных средств (низкая растворимость и химическая стабильность данных лекарственных веществ) и преимущества липосом для их доставки. Установлено, что наиболее перспективной технологией получения липосом с лекарственными веществами, обладающими антиоксидантными свойствами, является метод гидратации тонкой пленки с дальнейшим озвучиванием.

Ключевые слова: антиоксиданты, липосомы, фосфолипиды, направленный транспорт, метод гидратации тонкой пленки, ультразвуковое воздействие.