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"QUALITY BY DESIGN" IN LIPOSOMAL DRUGS CREATION

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Nanobiotechnological preparations creation is one of the promising areas of modern pharmacy, since it allows creating products of a qualitatively new level. The procedure development, based on an understanding of the product characteristics and the technological process, confirmed by reliable scientific data.

The article is devoted to the pharmaceutical development of liposomal drugs. On the basis of our own experience in the development of liposomal medicinal forms, as well as on the basis of literature data, the main components in their composition were detected and these components impact on the quality indicators of liposomes were studied. Individual lipids function in nanoparticle membrane and their interaction, which determines the stability both in the technological process and upon storage of the product, were considered. The advantages and disadvantages of cholesterol incorporation into liposomes with hydrophilic and hydrophobic active pharmaceutical ingredients were described. Cryoprotectors and buffer systems role in ensuring nanopreparation stability is discussed.

Key words: liposomes, phospholipids, cholesterol, cryoprotector, buffer system, Quality by Design.

The wide interest shown today to nanobiotechnological products, in particular to liposomal (Ls) drugs, in pharmacy is quite understandable — these drugs, possessing a wide spectrum of action, are intensively used for diseases of various etiologies diagnostics, prevention and treatment [1–6]. The Ls drugs creation is one of the promising areas of modern nanopharmacology [7–9].

The number of Ls medicinal forms on the world pharmaceutical market is more than 50 drugs, 5 of which are developed and licensed in Ukraine. Research begun in Ukraine in the early 90s led to the creation of Ls drugs of various directions: Lipin (pulmonology, cardiology, nephrology, obstetrics and gynecology); Lipodox (oncology); Lioliv (hepatoprotector) and two forms of Lipoflavone, namely eye drops (ophthalmology) and an injection form (cardiology) [10–14]. The indicated Ls preparations have found wide application in clinics of Ukraine: Lipin [3, 4, 15–21], Lipodox [4, 22–25], Lioliv [4, 26, 27], Lipoflavone [28–31]. In subsequent years, a team of authors proposed technologies for obtaining Ls preparations containing various active pharmaceutical ingredients (API), studied their physicochemical and pharmacological properties: irinotecan [32–34], cytochrome *c* [35–37], coenzyme Q10 [38, 39], curcumin [40, 41], cisplatin [42, 43], docetaxel [44–46] and a number of other products, including complex Ls preparations containing several APIs [40].

The advantage of Ls drugs in comparison with the free API form in an equivalent concentration is a decrease in toxicity (anticancer drugs irinotecan hydrochloride, doxorubicin hydrochloride, platinum drugs, etc.), prolonged action of API when administered; increased bioavailability for lipophilic substances (quercetin, curcumin, antral, etc.).

The aim of the work was to analyze the pharmaceutical development of Ls drugs. This

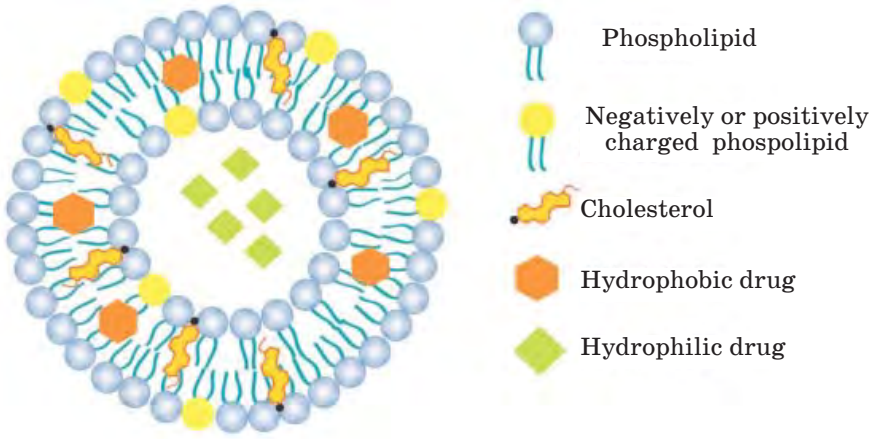

Fig. 1. **The structure of Ls preparations [47]**
In the developed preparations, hydrophobic substances are represented by quercetin, curcumin, phosphatidylcholine, etc., hydrophilic ones — by doxorubicin, irinotecan, oxaliplatin, etc.

report considered the issues of "Quality by design" in the creation of Ls drugs, namely the individual lipids role of nanoparticle membranes, their interaction, which determined the stability both in the process of technology and upon storage of the finished drug.

After analyzing the composition of the drugs proposed over the years, it should be noted that the Ls drugs include: API, phospholipids (PhL), cholesterol (Chol), polyethylene glycol derivatives (PEG), stabilizers (cryoprotectors); buffer systems providing the Ls structure and their physicochemical and pharmacological properties. The most important components of the Ls membrane are PhLs.

PhLs. The main membrane-forming components of Ls preparations are PhLs of various structure (natural and synthetic), differing in charge, saturation of fatty acids, properties of polar groups. To a certain extent, the effectiveness of Ls containing PhLs is based on the properties of the PhLs themselves [48–50].

The structure of PhL is characterized by the presence of hydrophobic and hydrophilic fragments in the composition of one molecule, as well as by the diversity of each of these fragments structure, which largely determines the role of PhL in a number of cellular processes [48]: structural function — the PhLs mixture should be able to form a stable bilayer for the functioning of membrane proteins (such PhLs include phosphatidylcholine (PC), phosphatidylglycerol (PG), sphingomyelin (SM), phosphatidylinositol (PI) and a number of others, and at the same time, there are some PhLs in the membrane that are capable of forming non-bilayer structures (phosphatidylethanolamine (PEA), diphosphatidylglycerol (DPG), phosphatidic acid (PA), etc.) The formation of such highly curved membrane sections is necessary during contact between membranes (cell fusion process) or when certain proteins are bound in the membrane, which ensures the existence of the membrane in a functionally active state. During hydration in the aqueous phase, PhLs spontaneously form Ls due to their thermodynamic phase properties [48–50]. When choosing a PhL component, it is necessary to take into account the phase transition temperature, i.e. the temperature at which PhLs pass from the gel to the liquid crystal phase. The phase transition temperature depends on the structure of the PhL molecule, the saturation of fatty acids and the structure of the polar groups. It should be borne in mind that natural PhLs containing different fatty acids in two positions have a non-standard phase transition temperature, especially since natural, for example, egg PC (EPC) is represented by the PC family containing a number of fatty acids [48] and does not have a clear phase transition temperature. PhLs with long chain fatty acids have a higher phase transition temperature. When using PhLs with a temperature below the phase transition temperature, they are in the gel phase, which in turn gives Ls low fluidity and permeability. When Ls is obtained at a temperature higher than the phase transition temperature, the PhLs are in the liquid crystalline phase, which provides greater fluidity and permeability. It should be noted that such a structure can simultaneously impede the penetration of hydrophilic APIs through the Ls membrane.

An independent question is PhL selection for the basis of Ls. When choosing a PhL, we

were guided by a number of requirements: the maximal API inclusion in the bilayer or in the aqueous phase of Ls, Ls stability both upon obtaining the Ls emulsion and upon hydration after lyophilization, storage stability, absence of undesirable reactions upon introduction into the body, etc. Both natural (EPC, hydrogenated soy PC (HSPC), sunflower PC (SFPC)) and synthetic (dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC) and other have been studied previously. According to a number of researchers, the use of unsaturated PhLs should be avoided due to the fact that these lipids are subject to peroxidation processes. In our opinion, it is possible to use unsaturated PhLs, in particular EPC, and the use of this lipid has a number of significant advantages, including economic ones [4]. To prevent peroxidation of EPC, a number of protective measures are used: a) nitrogen or argon was used at the stage of lipid film obtaining and its hydration, and the process of obtaining Ls was carried out in a protective atmosphere of these gases; b) limiting illumination; c) in cases where it is possible, at the technological stages the storage mode of the liquid preparation at a temperature of 2–6 °C was used; d) Ls storage after lyophilization throughout the entire period at a temperature of about minus 10 °C; e) sealing of lyophilized preparations was carried out in a protective gas atmosphere [4]. The API used can also determine the choice of one or another PhL. At the same time, in our opinion, the influence of the API structure does not significantly affect the choice of PhL. Thus, when obtaining Ls forms of anthracyclines, several medicinal Ls forms have been created that do not differ in pharmacological action, the composition of which is different: Doxil (HSPC:Chol:PEG–2000–DSPE 56:38:5); Myocet (EPC:Chol 1:1); Daune–Home (DSPC: Chol 2:1); Lipodox (EPC:Chol 0.85:0.15). As can be seen from the data presented, various PhLs have been introduced into the Ls forms of anthracyclines: EPC, HSPC, DSPC. When determining PhL for Ls formation, it is imperative to study the effect of the molecule structure and its charge on the properties of nanoparticles, unsaturation degree of fatty acid residues and products of PhL peroxidation, and other factors [4, 33, 34].

PhL is used in the Ls forms: neutral (various PC), anionic (DPG, PG, PA, PI) and cationic (PEA, dioleylphosphatidylethanolamine (DOPE), 1,2-dioleyloxy-3- [trimethytlamine]-propane (DOTAP)). For example, in the composition of the drug Visudin (EPG); DepoCyt (DPPG); hepatitis A vaccine (DOPE). In previous studies, anionic PhLs (PI, DPPG, DPG) were used [4, 35, 36, 39, 45, 46]. The studies carried out on the anionic PhLs inclusion in the Ls membrane bilayer when obtaining the Ls forms of cytochrome c, docetaxel, ubiquinone, oxaliplatin, curcumin, etc. have shown the following advantages: an increase in the inclusion of an active substance in Ls; stabilization and standardization of Ls emulsion; increasing the manufacturability of the process due to increasing the filtration rate of the Ls emulsion; the stability of the Ls emulsion upon rehydration of the lyophilized preparation [4, 46, 51]. In addition, the introduction of anionic PhLs made it possible to prolong the effect of the drug. For example, DPPG inclusion from EPC into the membrane allows increasing the percentage of cytochrome c incorporation into Ls due to the interaction of negatively charged DPPG with positively charged cytochrome c, which is based on the specific cytochrome c interaction with anionic PhLs [35–37, 52]. The effectiveness of cytochrome c Ls form has been shown in a number of pharmacological models [36, 37, 53]. It should be noted that there is no unequivocal opinion on the use of negative and positive PhLs. Using cytochrome c as an example, some authors confirm the effectiveness of anionic PhLs, others — of cationic PhLs [54–59]. Complex Ls preparations were proposed containing two phenolic antioxidants: curcumin and quercetin. When creating the drug, several PhLs were studied: EPC, HSPC and SFPC. The optimal relationships between bioflavonoids and PC have been determined. Moreover, EPC was the most effective. Chol inclusion in the Ls composition did not lead to an improvement in curcumin and quercetin incorporation into nanoparticles and Ls stabilization. At the same time, DPPG introduction into Ls made it possible to increase the percentage of API inclusion in Ls, maintain uniformity of dimensions and improve the manufacturability of the production process. A synergistic effect of antioxidants complex has been shown [40, 60]. Ls compositions of antioxidants were obtained, in which the complex of quercetin and curcumin/quercetin and ubiquinone was introduced. The incorporation of hydrophobic antioxidants into the Ls bilayer was no less than 85–95 %. The preparations were lyophilized, the size of nanoparticles in which during hydration was no more than 300 nm. A high antioxidant activity has been demonstrated, and each of the AFIs used in model experiments on animals acted on different markers of oxidative stress [61].

It should be noted that it was proposed to introduce Chol into a number of Ls drugs.

Chol. When analyzing the Chol content in Ls preparations, attention is drawn to the fact that Chol is mainly contained in products containing hydrophilic APIs (Doxil, DepoCyt, Lipodox, Ls form of irinotecan, etc.). Chol is a critical component [62] both in the formation of Ls and in the release of hydrophilic molecules from Ls. Chol influences the fluidity and permeability of the Ls membrane from EPC.

Chol was used to develop Ls medicinal forms containing hydrophilic APIs: doxorubicin and irinotecan. Both medicinal forms were obtained using the lipid film method, high pressure homogenization and the pH gradient method. When studying the dependence of irinotecan encapsulation degree on the Ls composition, the inclusion of API was found: EPC (100 wt %) — 33%; EPC:Chol (95:5 wt %) — 44%; EPC:Chol (85:15 wt %) — 57%; EPC:Chol (70:30 wt %) — 71%. As the Chol content increases, the degree of irinotecan encapsulation increases [33]. However, at an EPC:Chol ratio of 70:30 wt %, the membrane rigidity increases, which requires an increase in pressure when sterilizing filtration is used. Also, during the analysis of the particle size by laser diffraction, the presence of particles with a diameter of more than 5 μm was detected, which indicates the inhomogeneity of the emulsion, and also affects the possibility of sterilizing filtration. Based on the data obtained, the EPC:Chol ratio of 85:15 wt % was selected for further experiments. The experiment has showed that membranes made of EPC (100%), EPC: Chol (95: 5) do not have a high degree of encapsulation, and are also unstable upon lyophilization using various cryoprotectors. It has been also shown that Ls above 220 nm are absent. The Ls membrane based on EPC:Chol (85:15) shows a high degree of encapsulation and stability upon lyophilization. Trehalose at a concentration of 6.0% was used as a cryoprotector. Similar data were obtained during the development of doxorubicin Ls form [33]. Chol decreased the release of doxorubicin from Ls and, at the same time, the antitumor activity increased. It has been found that Chol inclusion in Ls can lead to an increase in the size of particles obtained by lyophilization, as well as a decrease in the amount of API included in Ls. Apparently, Chol presence decreases the rate of hydrophilic APIs passage into Ls and hydrophilic APIs escape from Ls. The composition of the preparation also determines the technological parameters of LS preparations lyophilization. It has been established that slow Ls freezing leads to an increase in the percentage of API inclusion after lyophilization and rehydration, in comparison with fast freezing [63]. Thus, "rigid" Ls containing Chol retained their structure to a greater extent during slow freezing than during fast freezing. API and PhL composition of Ls determines the need in Chol for nanoparticles. The Ls preparations developed by us with lipophilic APIs contain EPC as the main PhL, in which unsaturated fatty acids are mainly represented. Probably, lipophilic components forming lipid bilayer lead to membrane "rigidity" and do not require a steroid component in Ls. Also, it should be noted that Chol introduction into Ls with lipophilic components significantly complicates the sterilizing filtration process of the emulsion through membranes with a pore size of no more than 0.22 μm, which may be associated with an increase in the "rigidity" of the Ls membranes. Due to the fact that Chol presence in Ls did not lead to an increase in API inclusion in nanoparticles and worsened the technological parameters of Ls obtaining, it was decided to reject of sterols use for lipophilic substances: quercetin (Lipoflavon), antral (Lioliv), curcumin, coenzyme Q10 and others [11, 13, 14, 38, 39, 60]. It should also be noted that the presence of Chol high content in the Ls membrane led to a decrease in the penetration rate of the hydrophilic medicinal substance (anthracycline antibiotics — doxorubicin, epirubicin, idarubicin; fluorouracil, platinum preparations, irinotecan) into nanoparticles [4, 33, 42].

Cryoprotectors. The proposed Ls preparations are presented as lyophilized forms, which makes it possible to stabilize nanosize and provide a longer shelf life. To preserve the nanosize during lyophilization, it is necessary to introduce cryoprotectors into the Ls composition. When choosing a cryoprotector and determining its concentration, we were guided by the following requirements: preservation of Ls nanosize during the storage period; the product should be readily soluble when administered to humans; have no impact on the physicochemical and pharmacological properties of Ls; have no effect on the technological parameters of Ls obtaining. Disaccharides lactose monohydrate and trehalose dihydrate in various concentrations were introduced as cryoprotectors into the Ls preparations being developed. The amount of disaccharides in the proposed preparations is different [4, 10–14, 32, 35, 38, 45, 60, 63]. The content of cryoprotectors in the Ls emulsion is from 2.5% to 6.0%, depending on the composition of the preparation, which ensured the safety of Ls nanosize during lyophilization and storage.

Buffer systems — pH regulators. A phosphate saline buffer was used as the buffer solution. This buffer system is non-toxic and is widely used in the production of pharmaceuticals. For the technology of the pH gradient with API inclusion, in addition to phosphate saline buffer, citrate was used, which is the part of Ls form and is contained in the human body (tricarboxylic acid cycle). These buffer systems are widely used in pharmaceutical technology as pH stabilizers [64]. The use of buffer mixtures makes it possible to stabilize the pH of both the nanoparticles themselves and the APIs included in their composition. The pH of Ls preparations ranged from 5.0 to 7.4 [4, 12, 14, 32, 33, 35, 38, 46].

The analysis of the data obtained during the Ls drugs creation suggests that the development of "Quality by design" of the specified form of drugs requires a variety of experimental works: determination of the PhL components optimal ratio in the lipid membrane and their concentration, PhL charge and their fatty acid composition; study of cryoprotector type and its content in the preparation; pH value and ionic strength of the buffer system, etc. An independent question is the determination of the need for Chol inclusion in the Ls membrane, taking into account its role in increasing the lipid structure rigidity, which can lead to the appearance of particle size heterogeneity both during Ls production and during lyophilization. In addition, the presence of Chol can reduce the effectiveness of sterilizing filtration. The composition of Ls lipid membrane is also determined by API structure and its content in the preparation. Ls drug design requires experimental determination of drug sublimation modes, nanoparticle formation method and technological procedures of API loading into Ls.

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"QUALITY BY DESIGN" ПРИ СТВОРЕННІ ЛІПОСОМАЛЬНИХ ЛІКАРСЬКИХ ПРЕПАРАТІВ

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Створення нанобіотехнологічних препаратів є одним з перспективних напрямів сучасної фамації, оскільки дає змогу створювати продукти якісно нового рівня. Стратегія «Quality by Design» передбачає системний підхід до фармацевтичної розробки, що грунтується на розумінні особливостей продукту і процесу його отримання, підтверджених надійними науковими даними.

Статтю присвячено фармацевтичній розробці ліпосомних лікарських препаратів. На основі власного досвіду, а також даних літератури з розробки ліпосомних лікарських форм визначено основні компоненти, що входять до їхнього складу, і встановлено вплив кожного з них на показники якості ліпосом. Розглянуто роль окремих ліпідів мембрани наночастинок, їх взаємодію, що визначає стабільність як у процесі технології, так і під час зберігання готового препарату. Описано переваги і недоліки введення холестеролу до складу ліпосом з гідрофільними та гідрофобними активними фармацевтичними інгредієнтами. Обговорюється роль кріопротекторів і буферних систем у забезпеченні стабільності нанопрепарату.

Ключові слова: ліпосоми, фосфоліпіди, холестерол, кріопротектор, буферна система, Quality by Design.

"QUALITY BY DESIGN" ПРИ СОЗДАНИИ ЛИПОСОМАЛЬНЫХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ

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Создание нанобиотехнологических препаратов является одним из перспективных направлений современной фармации, поскольку позволяет создавать продукты качественно нового уровня. Стратегия «Quality by Design» предполагает системный подход к фармацевтической разработке, основанный на понимании особенностей продукта и процесса его получения, подтвержденных надежными научными данными.

Статья посвящена фармацевтической разработке липосомальных лекарственных препаратов. На основе собственного опыта, а также данных литературы по разработке липосомальных лекарственных форм определены основные компоненты, входящие в их состав, и установлено влияние каждого из них на показатели качества липосом. Рассмотрена роль отдельных липидов мембраны наночастиц, их взаимодействие, определяющее стабильность как в процессе технологии, так и при хранении готового препарата. Описаны преимущества и недостатки введения холестерола в состав липосом с гидрофильными и гидрофобными активными фармацевтическими ингредиентами. Обсуждается роль криопротекторов и буферных систем в обеспечении стабильности нанопрепарата.

Ключевые слова: липосомы, фосфолипиды, холестерол, криопротектор, буферная система, Quality by Design.