

ТЕХНОЛОГІЯ ЛІКАРСЬКИХ ПРЕПАРАТІВ

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THE STUDY OF THE LIPID MEMBRANE CHARGE EFFECT WHEN CREATING LIPOSOMES WITH OXALIPLATIN

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The fight against cancer diseases is one of the most urgent problems of modern pharmacy. One of the basic standards of treatment is surgery in order to remove tumours with chemotherapeutic agents for suppression of disease manifestations. One of the ways for reducing toxicity of cytostatics is their incorporation into liposomes – nanoparticles composed of the lipid bilayer surrounding the internal cavity with the aqueous medium. Oxaliplatin is a platinum-containing chemotherapeutic agent of the 3-rd generation used as monotherapy or in combination with other drugs. When creating liposomal drugs the beginning of the work is associated with the study of the composition of the lipid membrane. The aim of the work is to study the effect of the lipid membrane charge when creating liposomes with oxaliplatin. Four types of differently charged lipid membranes for liposomal oxaliplatin formulation have been tested. Liposomes were formed by the lipid layer method with further high pressure homogenization. As a criterion the encapsulation degree was used. The highest encapsulation degree has been determined in negatively charged liposomes with the lipid membrane modified by dipalmitoyl phosphatidylglycerol (DPPG).

Fighting cancer diseases is one of the primary problems of modern pharmacy [5]. One of the basic standards of treatment is surgery in order to remove tumours with chemotherapeutic drugs for suppression of the disease exacerbation [8]. The question concerning creation of new drugs and more modern dosage forms of substances used in practice currently is still open.

One of the disadvantages of the use of cytotoxic drugs is their side effects that adversely affect the heart and nervous system and the overall health [3]. One of the ways for reducing toxicity of cytostatics is their incorporation into liposomes – nanoparticles composed of the lipid bilayer surrounding the internal cavity with the aqueous medium inside. Liposomal cytostatics have the lower level of cardiotoxicity, less myelosuppression and less severe other side effects [4, 7].

Oxaliplatin is a platinum-containing chemotherapeutic agent of the 3-rd generation used as monotherapy or in combination with other drugs in oncology [6].

The research literature provides some examples of developing the liposomal form of oxaliplatin, but no one experiment have led to creation of a reliable technology, and the industrial production of the drug [1]. The registered liposomal oxaliplatin is still absent at the market. The authors had a task to create the industrial technology for manufacturing of liposomal oxaliplatin. When creating liposomal drugs the beginning of the work is associated with the study of the composition of the lipid

membrane. The lipid composition will influence on the quality of a future pharmaceutical product and prospects of its development into a finished dosage form.

Oxaliplatin is a complex molecule, which consist of the central atom of platinum surrounded by two ligands – oxalate and diaminocyclohexane (Fig. 1). Thus, the electron density in the molecule is distributed in such effect that oxygen atoms bound to the platinum atom bear a negative charge, and the platinum atom, consequently, is positively charged. Such structure explains the complexing properties of oxaliplatin in relation to both the positively and negatively charged molecules.

The aim of the work was to study the effect of the lipid membrane charge when creating liposomes with oxaliplatin

Materials and Methods

To manufacture liposomes egg phosphatidylcholine (Lipoid, Germany), cholesterol (Sigma-Aldrich, USA), dioleoyl trimethylammonium propane (as a chloride salt), dipalmitoyl phosphatidylethanolamine, dipalmitoyl phosphatidylglycerol (AvantiPolarLipids, USA) were used. The lipid film was obtained by the evaporation method on a Buchi Rotovap R-210 rotary evaporator with a vacuum controller, the residual pressure was 15 mbar. For homogenization a "Sapphire" ultrasonic bath with the volume of 2.8 L and the operating frequency of 35 kHz of the ultrasound generator and the power of 130 W, as well as a Microfluidics Microfluidizer M-110P extruder

Table

The comparative data of the encapsulation degree for liposomal oxaliplatin with a different composition in the lipid membrane

Composition of the lipid membrane	The average size of liposomes, nm	Liposome surface charge	Oxaliplatin encapsulation ($p = 0.05; n = 5$), %
Phosphatidylcholine/cholesterol 80/20	102	Neutral charge	10.12±0.13
Dipalmitoyl phosphatidylethanolamine/ phosphatidylcholine/cholesterol 5/75/20	Liposomes are not formed		
Phosphatidylcholine/cholesterol/dioleoyl trimethylammonium propane (as a chloride salt) 60/20/20	148	Positive charge	8.94±0.15
Phosphatidylcholine/cholesterol/dipalmitoyl phosphatidylglycerol 70/20/10	131	Negative charge	15.87±0.14

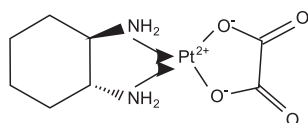


Fig. 1. The structure of oxaliplatin.

were used. The size of liposomes was determined by the laser diffraction method using a “Malvern Instruments Zetasizer Nano ZS” device. The encapsulation degree was determined by HPLC in the gel filtration version on a Shimadzu LC-20 instrument.

Results and Discussion

According to the ACD Labs programme the protonation constant – pKa of oxaliplatin is 5.88, and it can be described as practically neutral. The structure of oxaliplatin in the absence of protonated amino groups makes it impossible to use the “chemical gradient” method. Since oxaliplatin is a hydrophilic substance and practically insoluble in a non-polar organic solvent, it is impossible to apply the technology of its common dissolution with lipids with the subsequent formation of the lipid film. Some technologies based on sorption of oxaliplatin molecules on the liposomal membrane were tested. The experiment consisted of 3 research steps for developing the composition of the lipid membrane – neutral lipid membrane, positively charged lipid membrane and negatively charged lipid membrane.

STEP 1

As the primary model for liposomal oxaliplatin the neutral composition of the lipid membrane was selected.

The lipid film was obtained with the mass ratio of phosphatidylcholine/cholesterol of 80/20. The lipid film obtained was hydrated by oxaliplatin solution with the concentration of 2 mg/ml. The concentration of lipid in the emulsion was 20 mg/ml. Extrusion was carried out in 9 cycles at 900 atm. The average size of liposomes was 102 nm, 200 nm particles were absent. Inclusion of oxaliplatin in liposomes was performed using the HPLC method developed [2]. Encapsulation of oxaliplatin into liposomes was 10.12%. The results are given in Table.

Earlier experiments show that the volume entrapped into liposomes by the “passive encapsulation” during formation and extrusion is at the level of 9-11% (vol). Therefore, we can assume that the resulting encapsula-

tion approximately corresponds to the internal volume of the liposomes formed. The data suggest that encapsulation is based on the principle of the capacitive capture of oxaliplatin by the internal cavity of the liposomes formed. However, sorption of the active substance on liposomes surface does not occur.

As part of STEP 1 the experiment in obtaining liposomes with addition of dipalmitoyl phosphatidylethanolamine as a modifier of the lipid membrane in proportion of dipalmitoyl phosphatidylethanolamine/phosphatidylcholine/cholesterol (5:75:20%) was performed.

The lipid sample was dissolved in a mixture of anhydrous ethanol – chloroform (1:1) at 40°C with the treatment in an ultrasound bath for 10 min. Then the lipid film was formed by evaporation. The resulting film was hydrated with oxaliplatin solution with the concentration of 2 mg/ml. The total lipid concentration was 20 mg/ml. However, we failed to obtain an emulsion because of formation of a flaky precipitate. Therefore, the conclusion was made about irrationality of using dipalmitoyl phosphatidylethanolamine as a lipid film modifier for production of liposomal oxaliplatin. The composition of the membrane and characteristics of liposomes are given in Table.

STEP 2

At STEP 2 cationic liposomes with the composition of lipid phosphatidylcholine/cholesterol/dioleoyl trimethylammonium propane (as a chloride salt) in the mass ratio of 60/20/20 were used. The lipid sample was dissolved in ethanol, and then the lipid film was obtained. After formation the lipid film was hydrated with oxaliplatin solution with the concentration of 2 mg/ml. The total concentration of lipids in the emulsion was 20 mg/ml. Extrusion was carried out in 6 cycles at 900 atm. The average size of liposomes was 148 nm. Encapsulation of oxaliplatin into liposomes after production was 8.94%. Proximity of the encapsulation degree to the internal volume of liposomes gives reason to believe that in this membrane composition a capacitive capture of oxaliplatin by liposomes takes place without the presence of sorption effects. In Fig. 2 the results of measurement of the size of the liposomes obtained by the laser diffraction method are shown. The composition of the characteristics of membranes and liposomes are shown in Table.

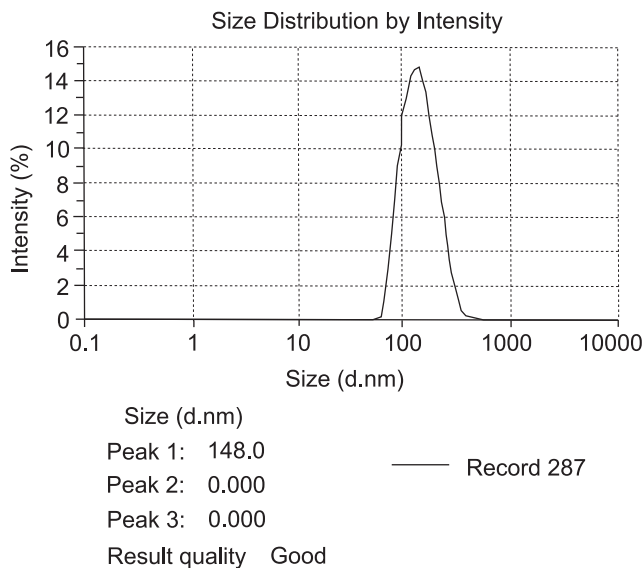


Fig. 2. The results of measuring the size of oxaliplatin liposomes with the lipid membrane composition: phosphatidylcholine/cholesterol/dioleoyl trimethylammonium propane (as a chloride salt) with the mass ratio of 60/20/20.

STEP 3

In the experiments of STEP 3 a negatively charged lipid – dipalmitoyl phosphatidylglycerol was used as modifier of the lipid membrane. Lipids with the composition of phosphatidylcholine/cholesterol/dipalmitoyl phosphatidylglycerol in the mass ratio of 70/20/10 were dissolved in chloroform to obtain a lipid film. Then the film was hydrated with oxaliplatin solution with the concentration of 2 mg/ml for complete emulsification of lipids. The concentration of lipid in the emulsion was 20 mg/ml. Extrusion was carried out in the mode of 900 atm.

To obtain liposomes with an average size of 131 nm eight cycles of homogenization was applied. The encapsulation degree of oxaliplatin into liposomes immediately after preparation was studied. Encapsulation was 15.87%, which might indicate an additional mechanism of sorption during preparation of liposomal oxaliplatin with dipalmitoyl phosphatidylglycerol as a modifier. When forming the lipid film along with the passive en-

capsulation the mechanism of sorption, in which oxaliplatin sorbed by negatively charged liposomes modified by dipalmitoyl phosphatidylglycerol, is probable.

It may be assumed that there is the interaction of negatively charged hydrophilic parts of dipalmitoyl phosphatidylglycerol with a positively charged platinum atom in the oxaliplatin molecule (Fig. 1).

Table shows the comparative data concerning the study of different types of membranes for development of liposomal oxaliplatin by the sorption technology.

The data in Table demonstrate that liposomes with neutral and positively charged membranes have encapsulation degrees of 9-10%, which are comparable with the passive inclusion of oxaliplatin when forming liposomes. However, negatively charged liposomes have the encapsulation degree of 15.87%. It indicates the presence of an additional mechanism, along with the passive encapsulation (probably sorption), which increases the total encapsulation of oxaliplatin into liposomes. Based on the data obtained dipalmitoyl phosphatidylglycerol is a prospective modifier for the lipid membrane of liposomal oxaliplatin. It can be selected for further work in creating low toxicity liposomal drug with oxaliplatin.

CONCLUSIONS

1. Four different types of liposomal oxaliplatin with the different lipid composition have been created and studied. The size of the liposomes is in the range of the 100-150 nm.

2. It has been proven that depending on the charge of the membrane the encapsulation degree of oxaliplatin into liposomes varies. Oxaliplatin sorption on the surface of the lipid membrane has the greatest effect on the encapsulation degree.

3. Liposomes with dipalmitoyl phosphatidylglycerol as a modifier (anionic liposomes) have the greatest degree of encapsulation of the active ingredient among the membranes tested. Encapsulation for anionic liposomes is $15.87 \pm 0.14\%$ compared to $10.12 \pm 0.13\%$ for neutral liposomes and $8.94 \pm 0.15\%$ for cationic ones at $p = 0.05$ and $n = 5$.

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ВИВЧЕННЯ ВПЛИВУ ЗАРЯДУ ЛІПІДНОЇ МЕМБРАНИ ПРИ СТВОРЕННІ ЛІПОСОМ ІЗ ОКСАЛІПЛАТИНОМ

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Ключові слова: ліпосоми; оксаліплатин; ліпідна плівка; гомогенізація методом високого тиску

Боротьба з онкологічними хворобами є однією з актуальних задач сучасної фармації. Одним з основних стандартів лікування є хірургічне втручання для видалення новоутворення з подальшим застосуванням хімотерапевтичних засобів для супресії повторних проявів хвороби. Одним зі шляхів зниження токсичності цитостатиків є включення їх у ліпосоми – наночастки, які складаються з ліпідного бішару, що оточує внутрішню порожнину з водним середовищем всередині. Оксаліплатин – платиновмісний хімотерапевтичний агент третього покоління, який використовується як в монотерапії, так і в комбінації з іншими лікарськими засобами. Початок роботи при створенні ліпосомальних препаратів пов'язаний із вивченням складу ліпідної мембрани. Мета роботи – вивчення впливу заряду ліпідної мембрани при створенні ліпосом із оксаліплатином. Для створення ліпосом із оксаліплатином було апробовано 4 типи мембран із різним зарядом поверхні. Ліпосоми отримували методом ліпідної плівки з подальшою гомогенізацією методом високого тиску. Порівняння проводили за ступенем інкапсуляції. Найбільший ступінь інкапсуляції було визначено у негативно заряджених ліпосом з ліпідною мембраною, модифікованю дипальмітоїлфосфатидилгліцерином.

ИЗУЧЕНИЕ ВЛИЯНИЯ ЗАРЯДА ЛИПИДНЫХ МЕМБРАН ПРИ СОЗДАНИИ ЛИПОСОМ С ОКСАЛИПЛАТИНОМ

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Ключевые слова: липосомы; оксалиплатин; липидная пленка; гомогенизация методом высокого давления

Борьба с онкологическими болезнями – одна из актуальных задач современной фармации. Одним из основных стандартов лечения является хирургическое вмешательство для удаления новообразования с применением химиотерапевтических средств для супрессии проявлений болезни. Одним из путей снижения токсичности цитостатиков является включение их в липосомы – наночастицы, состоящие из липидного бислоя, окружающего внутреннюю полость с водной средой. Оксалиплатин – платиносодержащий химиотерапевтический агент 3-го поколения, который используется как в монотерапии, так и в комбинации с другими лекарственными средствами. Начало работы при создании липосомальных препаратов связано с изучением состава липидной мембраны. Цель работы – изучение влияния заряда липидной мембраны при создании липосом с оксалиплатином. Для создания липосом с оксалиплатином были апробированы 4 типа мембран с различным зарядом поверхности. Липосомы получали методом липидной пленки с последующей гомогенизацией методом высокого давления. Сравнение проводили по степени инкапсуляции. Наибольшая степень инкапсуляции была определена в отрицательно заряженных липосомах с липидной мембраной, модифицированной дипальмитоилфосфатидилглицерином.