VOLGOGRAD STATE MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY AND BIOINFORMATICS

Methodological recommendations for students for practical classes
«Immunobiological and gene therapy drugs»

Thematic block: **Immunobiological drugs**

Class topic:

Vectors as carriers for gene delivery. Main types, principles of action and characteristics of viral vectors (capacity, selectivity, duration of gene expression, immunogenicity, ease of production, possibility of integration into DNA cells, probability of the patient having antibodies). Physical and chemical methods of delivering vectors based on viruses (gene gun, electroporation, magnetofection, sonoporation, using various nanoparticles - silicon, gold, calcium phosphate, lipids).

Pharmaceutical faculty

1. Class aims

• learn to analyze and classify the action of immunobiological agents based on the totality of their pharmacological properties, mechanisms and localization of action;

• learn to evaluate the possibilities of using immunobiological agents for adequate pharmacotherapy.

2. TASKS:

1. For drugs with a vector delivery system, study:

• the main types, principles of action and characteristics of viral vectors (capacity, selectivity, duration of gene expression, immunogenicity, ease of production, the possibility of integration into DNA cells, the likelihood of the patient having antibodies);

• physical and chemical methods of delivering vectors based on viruses (gene gun, electroporation, magnetofection, sonoporation, using various nanoparticles - silicon, gold, calcium phosphate, lipids);

• classification of drugs;

• their use in medical practice.

3. THE FOLLOWING PRACTICAL SKILLS ARE PRACTISED DURING THE LESSON:

- ability to evaluate the possibilities of using various immunobiological agents based on ideas about their properties;

- ability to analyze the action of human albumin preparations; human immunoglobulin preparations; blood clotting factor preparations containing one of the blood clotting factors or a combination of them, based on the totality of their pharmacological properties, mechanism and localization of action.

4. ORDER OF CONDUCTING CLASSES:

Venue: classroom of the Department of Pharmacology and Bioinformatics.

Time: 2 AH

Competencies to be developed: УК-1.1.3, УК-1.2.1, УК-1.2.2, УК-1.2.3., УК-1.3.1, УК-1.3.2., УК-6.1.1., УК-6.2.1, УК-6.2.2, УК-6.3.1, УК-6.3.2, УК-6.3.3, УК-6.3.4, ОПК-1.1.1., ОПК-1.2.1, ОПК-1.2.2., ОПК-1.3.1, ОПК-6.1.1, ОПК-6.2.1, ОПК-6.3.1, ПК-7.1.1, ПК-7.2.1, ПК-7.3.1.

4.1 Technological map of the lesson

Part	№	Class stage	Time
1	1	Checking the students present at the lesson, lesson mode, lesson topic.	5 min
	2	Checking the initial level of students' knowledge (written survey).	10 min
	3	Survey on the topic of the lesson.	45 min
	4	Independent work of students (on prescriptions with analysis of the most complex prescriptions (if any in the topic), analysis of errors in medical prescriptions written by students; work with synonyms).	15 min
	5	Checking independent work	5 min
	6	Summing up the lesson. Assignment for the next lesson.	5 min
	7	Cleaning of workplaces.	5 min

4.2 Демонстрации
Демонстрация слайдов по данной теме при опросе по теме занятия.

4.2 Demonstrations
1. Demonstration of advertising brochures on this topic during a survey on the topic of the lesson.

4.3 **Lesson plan**

4.3.1 Introductory remarks by the teacher.

4.3.2 Analysis of theoretical material.

4.3.3 Conducting the test

Plan for analyzing theoretical material.

Vectors as carriers for gene delivery.

A vector is a nucleic acid molecule, most often DNA, used in genetic engineering to transfer genetic material into a cell, including into a cell of a living multicellular organism in vivo. Two main vectors are used to introduce recombinant DNA: plasmids and bacteriophages.

Vectors are classified depending on the hosts they are to enter (bacterial, yeast, etc.), depending on the type of replicon (plasmid, viral, combined, shuttle, artificial chromosomes) and depending on the functional purpose (integrative, expression, vectors for cloning, sequencing or transcription). You can select and order suitable vectors for your experiments by accessing the AddGene database or catalogs of commercial companies, and if something in the ready-made vectors does not suit you, you can modify them yourself.

In most cases, viruses are used as carriers for gene delivery, whose natural ability to introduce their genetic material into host cells can be not only harmful but also beneficial. Such viruses are first modified using genetic engineering methods, removing most of the genes responsible for virulence, which prevents the virus from dividing uncontrollably and frees up space for insertion of the target gene that needs to be delivered to the cell. These carrier viruses protect the therapeutic gene from being broken down by blood enzymes, force the target cells to capture the virus, separate the nucleic acid from the viral particle, and transport it to its destination (usually the cell nucleus). A cell that has been inoculated with such a virus is infected - but not with a dangerous infection, but with a disease-curing gene - and therefore begins to synthesize a new protein that directs the cell along the path to recovery. Many different viral vectors are being developed because different diseases require the

delivery of genes to different organs or tissues, and different levels and durations of expression are required to achieve a therapeutic effect. Typically, the following characteristics are used when selecting or developing a suitable viral vector:

Емкость — длина ДНК целевого гена, который может быть помещен в вектор.
Селективность поглощения целевыми для данной терапии клетками и отсутствие экспрессии в тканях, где получаемый белок может вызвать токсичность (например, в сердце).
Продолжительность экспрессии гена.
Иммуногенность — влияние вектора на иммунный ответ.
Простота производства.
Возможность интеграции в ДНК клетки или способность существования в качестве стабильного элемента в ядре клетки без геномной интеграции.
Вероятность наличия у пациента антител против этого вектора в случае, если организм ранее встречался с подобным вирусом, — это снижает эффективность вектора.

Thus, the ideal vector should have:

• places for convenient insertion of DNA fragments;
• sufficient capacity;
• selective markers that allow detection of cells with this vector - both "empty" and with an "insert";
• DNA regions that ensure its maintenance as a separate replicon or integration of the cloned fragment into the host genome;
• DNA regions that ensure (if required) efficient expression of the inserted gene in the selected host.

Despite the current prevalence of virus-based vectors, genes can also be delivered by other physical and chemical methods: gene gun, electroporation, magnetofection, sonoporation, the use of various nanoparticles (silicon, gold, calcium phosphate, lipids), etc.

Physical methods of delivery of genetic material.

Along with viral and non-viral systems for delivery of genetic material, using the natural properties and functions of cell membranes to deliver nucleic acids, there is a group of methods whose main task is physical damage to the plasma membrane, leading to the penetration of foreign genetic material into the cytosol. A number of physical methods have proven themselves in biotechnology, where they are used to transform cell cultures. There are also reports of the use of some physical methods for gene transfer at the organism level.

A classic physical method for delivering genetic material is the so-called "gene gun", first used to transfer genes into plants. The method is based on bombarding cells or tissue with metal particles coated with DNA. The required speed is given to the particles by a current of carrier gas (usually helium) or by a high-voltage electric discharge. Microparticles of gold, tungsten or silver with a diameter of about 1 μm are used as a carrier. To achieve the required efficiency of genetic material transfer and reproducibility of results, precise standardization of all physical and technical parameters of the experiment (particle size, gas pressure, etc.) is necessary. Currently, gene guns are used in ovarian cancer research. An obvious disadvantage of gene guns is traumatization of target tissues, which often leads to the death of transfected cells. The permeability of the plasma membrane can also be increased by electrical discharges. This is the basis of the electroporation method, another classic physical method of gene transfer. Application of an electric field to the plasma membrane that is greater than its own electrical capacity causes a redistribution of charges on the membrane with the subsequent

formation of pores, which allows DNA molecules to diffuse into the cells. The field parameters are selected based on the physical properties of the target cell membrane.

Intradermal, intramuscular and intratumoral delivery of plasmid DNA using electroporation is described. The main obstacle to the use of electroporation in vivo is also the increased death of cells exposed to the electric field. A more gentle technique for plasma membrane permeabilization is sonoporation, which is the treatment of target tissue with ultrasound. To deliver genetic material to cells using sonoporation, DNA is immobilized on the surface of microbubbles and injected into the bloodstream, followed by the application of ultrasound to the projection of the target organ. The bubbles consist of a core (perfluorocarbons or sulfur hexafluoride) filled with gas (air, nitrogen, inert gas) and coated with lipids, proteins, or synthetic biopolymers. Circulating microbubbles, similar in size to erythrocytes (diameter 2–4 μm), respond to the action of ultrasound and release DNA, which diffuses into permeabilized cells. Sonoporation is used to deliver genetic material to the brain, kidneys, abdominal cavity, as well as muscle tissue, including the heart muscle, etc.

«Cell squeezing» is a method invented in 2013. It allows to deliver molecules into cells by «gently squeezing» the cell membrane. The method excludes the possibility of toxicity or incorrect targeting, as it does not depend on external materials or electric fields.

In rare cases, hydroporation is used to deliver genetic material in vivo — increasing membrane permeability due to a sharp change in hydrodynamic pressure. The pressure is created by injecting large volumes of DNA solutions in a short period of time. This effect increases the permeability of the capillary endothelium and forms pores in the plasma membrane of the surrounding parenchyma cells, through which DNA penetrates. This method is usually used for gene therapy of liver cells.

DNA microinjection — introduction into the nucleus of animal cells using thin glass microtubules (d = 0.1-0.5 μm). The disadvantage is the complexity of the method, a high probability of destruction of the nucleus or DNA; a limited number of cells can be transformed. Not for human use.

Particle based methods.

A direct approach to transfection is the gene gun, in which DNA is linked into a nanoparticle with inert solids (usually gold, tungsten), which is then “fired” directly into the nuclei of target cells. This method is used in vitro and in vivo to introduce genes, in particular, into muscle cells, for example, in a disease such as Duchenne muscular dystrophy. The size of gold particles is 1-3 μm. A very effective method for transfecting DNA is its introduction through liposomes - small, membrane-bound bodies that can fuse with the cellular cytoplasmic membrane (CPM), which is a double layer of lipids. For eukaryotic cells, transfection is more effective using cationic liposomes, because the cells are more sensitive to them. The process has its own name - lipofection. This method is considered one of the safest today. Liposomes are non-toxic and non-immunogenic. However, the efficiency of gene transfer using liposomes is limited, since the DNA they introduce into cells is usually immediately captured by lysosomes and destroyed.

Another method is the use of cationic polymers, such as diethylaminoethyl dextran or polyethyleneimine. Negatively charged DNA molecules bind to positively charged polycations, and this complex then penetrates the cell by endocytosis. DEAE-dextran changes the physical properties of the plasma membrane and stimulates the absorption of this complex by the cell. The main disadvantage of the method is that DEAE-dextran is toxic in high concentrations. The method has not become widespread in gene therapy.

Delivery using histones and other nuclear proteins. These proteins, containing many positively charged amino acids (Lys, Arg), in natural conditions help to compactly pack a long DNA chain into a relatively small cell nucleus.

Magnetofection is a method that uses magnetic forces to deliver DNA to target cells. First, nucleic acids (NA) are associated with magnetic nanoparticles, and then, under the influence of a magnetic field, the particles are driven into the cell. The efficiency is almost 100%, and they are clearly non-toxic. In just 10-15 minutes, the particles are registered in the cell - much faster than other methods.

Impalefection (impalefection; "impalement", literally "impalement" + "infection") is a delivery method using nanomaterials, such as carbon nanotubes and nanofibers. In this case, the cells are literally pierced with a bedding of nanofibrils. The prefix "nano" is used to denote their very small size (within billionths of a meter).

In addition, the possibility of delivering "naked" nucleic acids (naked RNA and DNA) is also being studied. Such methods of target gene delivery may theoretically have advantages over viruses, since their use is easier to mass-produce (nanoparticle-based vectors, for example, are much easier to produce on an industrial scale), and the risk of genotoxicity and immunogenicity here will also potentially be low. However, so far, chemical gene delivery methods are less specific and precise than viral delivery, and therefore less effective, and methods of physically introducing genes into cells cannot be used in in vivo therapy. So far, no drug based on non-viral gene delivery methods has been approved for use in humans.

Disadvantages of gene therapy

Gene therapy technologies are in their infancy, so naturally they have many “teething problems” and a huge scope for improvement. The main challenges that need to be addressed are listed below:

• complexity, labor intensity, high cost and, as a consequence, poor scalability of the technology, due to which gene drugs are still incredibly far from being widespread (and it is not a fact that they will get there soon);

• the exorbitant cost of such treatment, which follows from the previous point, due to which it is available only to a few;

• often serious adverse events of a new type, sometimes even leading to irreversible consequences (up to and including death). However, as experience accumulates, doctors are already learning to cope with them;

• insufficient observation period for the use of gene therapy, hence there is uncertainty about the long-term effect, as well as concerns about possible long-term consequences of the use of such drugs;

• imperfection of the technology for delivering target genes: viral vectors, which are most often used, are far from ideal. In addition to the aforementioned problems with non-target integration into the genome (characteristic of lentiviral and γ-retroviral vectors), there is also the risk of activation of the immune system (for AAV vectors), which reduces the efficiency of target gene delivery. Moreover, repeated introduction of the vector is not always possible.

However, these are not only problems: they are also potential “growth points” for new technology, where new ideas have a chance to be realized in the form of tangible benefits.

4.3.5. Independent work of students:

1. Work with the reference book "Synonyms of medicines", conduct a search and write out in the workbook synonyms of medicines on this topic.

4.3.6 Checking the students' independent work.
4.3.7 Summing up the lesson. Answering students' questions.
4.3.8 Concluding remarks by the teacher.

Составитель
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Приложение 1

Перечень рекомендуемой литературы, включая электронные учебные издания:

1. Харкевич Д. А. Фармакология : учебник / Харкевич Д. А. - 11-е изд., испр. и доп. - М. : ГЭОТАР-Медиа, 2015. - 755, [5] с. : ил. - Текст: непосредственный.

2. Харкевич, Д. А. Фармакология : учебник / Д. А. Харкевич. - 13-е изд. , перераб. - Москва : ГЭОТАР-Медиа, 2022. - 752 с. : ил. - ISBN 978-5-9704-6820-3. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970468203.html

3. Фармакология : учебник / под ред. Р. Н. Аляутдина. - 6-е изд. , перераб. и доп. - Москва : ГЭОТАР-Медиа, 2022. - 1104 с. - ISBN 978-5-9704-6819-7. - Текст : электронный //

ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970468197.html

4. Майский, В. В. Фармакология с общей рецептурой : учебное пособие / В. В. Майский, Р. Н. Аляутдин. - 3-е изд. , доп. и перераб. - Москва : ГЭОТАР-Медиа, 2017. - 240 с. - ISBN 978-5-9704-4132-9. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970441329.html

5. Аляутдин, Р. Н. Фармакология. Ultra light : учеб. пособие / Р. Н. Аляутдин. - Москва : ГЭОТАР-Медиа, 2012. - 584 с. - ISBN 978-5-9704-1985-4. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970419854.html

6. Онкология : учебник / М. И. Давыдов, Ш. Х. Ганцев [и др.]. - Москва : ГЭОТАР Медиа, 2020. - 920 с. : ил. - ISBN 978-5-9704-5616-3. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970456163.html

7. Онкология : учебник / под ред. С. Б. Петерсона. - 2-е изд. , перераб. и доп. - Москва : ГЭОТАРМедиа, 2018. - 288 с. : ил. - ISBN 978-5-9704-4704-8. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970447048.html

8. Онкология / под ред. Чиссова В. И. , Давыдова М. И. - Москва : ГЭОТАР-Медиа, 2014. - 1072 с. - ISBN 978-5-9704-3284-6. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970432846.html

9. Медицинская микробиология, вирусология и иммунология : Т. 1 : учебник / под ред. Зверева В. В. , Бойченко М. Н. - Москва : ГЭОТАР-Медиа, 2020. - 448 с. - ISBN 978-5-9704-5835-8. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970458358.html

10. Медицинская микробиология, вирусология и иммунология : Т. 2 : учебник / под ред. Зверева В. В. , Бойченко М. Н. - Москва : ГЭОТАР-Медиа, 2021. - 472 с. - ISBN 978-5-9704-5836-5. - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : https://www.studentlibrary.ru/book/ISBN9785970458365.html

11. Этиотропная терапия острых вирусных инфекций у детей : учеб. пособие для спец. 06010365 "Педиатрия" / Крамарь Л. В., Арова А. А., Желудков Ю. А. и др. - Волгоград : Изд-во ВолгГМУ, 2012. - 156 с. - Текст: непосредственный.

12. Иоанниди Е. А. Хронические вирусные гепатиты B, D и C : этиопатогенез, эпидемиология, клиника, лечение и профилактика : учеб. пособие / Иоанниди Е. А., Божко В. Г., Беликова Е. А., Александров О. В. ; ВолгГМУ Минздрава РФ. - Волгоград : Изд-во ВолгГМУ, 2016. - 71, [1] с. : табл. - Текст : электронный // ЭБС ВолгГМУ : электронно-библиотечная система. - URL: http://library.volgmed.ru/Marc/MObjectDown.asp?MacroName=%D5%F0%EE%ED%E8%F7_%E2%E8%F0%F3%F1_%E3%E5%EF%E0%F2%E8%F2%FB_2016&MacroAcc=A&DbVal=47

13. Kharkevitch D.A., Pharmacology / Kharkevitch D.A. - M. : ГЭОТАР-Медиа, 2008. - 672 с. - ISBN 5-9704-0264-8 - Текст : электронный // ЭБС "Консультант студента" : [сайт]. - URL : http://www.studentlibrary.ru/book/ISBN5970402648.html (дата обращения: 28.02.2020). - Режим доступа : по подписке.

Перечень профессиональных баз данных, информационных справочных систем, электронных образовательных ресурсов, рекомендуемых для подготовки:

1. http://vrachirf.ru/ - Информационный портал Врачи России
2. https://pharmarf.ru – информационный портал Фарма России
3. https://www.rlsnet.ru/ - РЛС (регистр лекарственных средств России) (информационная справочная система)
4. http://www.drugs.com - Информационная база о лекарственных препаратах (информационная справочная система)

5. https://grls.pharm-portal.ru/ - государственный реестр лекарственных средств.
6. http://elibrary.ru – Электронная база, электронных версий периодических изданий на платформе Elibrary.ru (профессиональная база данных)
7. http://www.consultant.ru/ – Справочно-правовая система «Консультант-Плюс» (профессиональная база данных)