

## DIRECTED DRUG DELIVERY SYSTEMS. BIOTECHNOLOGICAL ASPECTS

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Publications concerning methods of creation of the drug delivery systems based on liposomal forms and receptor-dependent endocytosis are summarized. The methods of use of the directed drug delivery systems in creation of anticancerogenical and antibacterial agents, vaccines and in gene therapy are examined.

**Key words:** directed drug delivery systems, pharmaceuticals, liposomes, endocytosis, anticancer agents, monoclonal antibodies.

Nowadays, the obligatory criteria that enable to use pharmaceuticals in clinical practice are the following: efficacy, safety and quality. These parameters are determined by the results of appropriate investigations, such as: bioequivalence, pharmacological, toxicological and clinical trials and *in vitro* investigations. Efficacy and safety of pharmaceuticals are related to efficacy of their action on a particular cellular target and to their ability to maintain a high level of *in vivo* activity.

While developing new pharmaceuticals, the researchers often don't pay sufficient attention to their targeted delivery. At the same time it is clear that only a therapeutic agent of high *in vitro* activity level, bioavailability and specificity can be effective. It should be mentioned that present knowledge about intracellular transport mechanisms and molecular organization of cell surface allows to develop new, more effective technologies of targeted drug delivery. Use of these methods is intended to raise the drugs action specificity and to decrease in that way their toxicity and the concentration of acting agents as well [1, 2].

Effective intracellular delivery of pharmaceuticals becomes particularly important in cancer therapy [1-3]. The main reasons that essentially restrict efficacy of antitumor therapy are low specificity as well as primary resistance of tumor cells to chemotherapy agents and their resistance acquired during the treatment.

Two main approaches to selectivity improving of pharmaceuticals based on directed transport of acting agents to target cells by

means of liposomal forms and receptor-dependent endocytosis (RDE) are discussed in the article.

### Liposomal drug formulations

Ability of liposomes to hold different substances practically without restrictions on their chemical nature and size of molecules gives an unique opportunity to solve some medical and biological problems. For example, a lot of pharmaceuticals are characterized by low therapeutic index because their medicinal concentrations don't differ a lot from toxic concentration. Otherwise a medical product can rapidly lose its activity under inactivating agents action at a time of injection into an organism. Inclusion of such medical products in liposomes could greatly increase their therapeutic efficacy. On the one hand, medical product contained in liposome is immuned against the negative factors by liposome membrane. On the other hand the same membrane doesn't let a toxic product to exceed its maximum permissible concentration in the organism's biological liquids. In this case liposome acts as a depository whereof a medical product is being released little by little in required doses and during required time period [4-5].

In terms of biological compatibility, liposomes are the ideal carriers of pharmaceuticals. Their advantages are the following: natural biocompatibility of liposome phospholipides, that are the main component of biomembrane lipid matrix; biodegradation to the products that can be utilized to water and carbon dioxide;

nontoxicity and nonantigenicity; good solubilization. Under definite conditions the cells could absorb liposomes; their membrane could conjugate to a cell membrane that leads to intracellular delivery of their content [6].

It ought to be noted that liposomes are not kept by such organs as heart, kidneys, brain and nervous system cells. This phenomenon allows to bring down significantly cardiotoxicity, nephrotoxicity and neurotoxicity of the important pharmaceuticals for anticancer therapy.

Disadvantage of liposomal forms is bad pharmacokinetical characteristics caused by instability of liposomes, their quick opsonization at intravenous introduction and further absorption by a reticuloendothelial system (RES). Thereat usually liposomal carriers can't be directed to those organs and tissues where pathological process takes place [7].

It is known that pharmacokinetics of drugs encapsulated in liposomes is determined by coordination of two following factors: speed of excretion of a liposomal agent from plasma (purification) and stability of liposomes bonding with drugs in bloodstream. This process depends on characteristics of a medical product and liposomal carrier, namely on liposomes size and their physical and chemical characteristics, on permeability of individual tissues, on the nature of bond between liposome and medical product [5, 8]. If liposome surface is made as hydrophilic one under use of covalent bonded polyethylene glycol (PEG), liposomes could be protected against a reticuloendothelial system. In its turn, this leads to extension of half-cycle of a medical product existence in bloodstream and to slow penetration of the medical product into a tumor tissue [9]. PEG coating also inhibits protein-dependent binding with the cells. Scheme of steric stabilized liposome is following: proteins couldn't reach a liposome surface because of osmotic overpressure in a membranous space; flexible chains of immobilized polymers such as PEG create overpressure.

Liposomes have great prospects for delivering cytotoxic pharmaceuticals selectively into tumor tissue. Thereby the researchers should concentrate on a mechanism of selective accumulation of liposomal forms of the cytostatic agents in tumor tissue. Physiology of solid tumors differs from that of the normal tissues by a whole series of substantial aspects [6, 10]. Tumor vesicles are often abnormal misshapen capillaries with the permeable walls and slowed blood flow in contrast with regular and well-ordered vascularization of

normal tissues. Tumor growth requires permanent growth of the new vesicles. These differences could make problems physiologically in cancer treatment. For example, hypoxia in the solid tumors causes resistance to radiotherapy and to some antitumor agents. However, these differences can be used for selective cancer treatment. Permeable blood vesicles can find application for use of liposomes with different structures as well as steric stabilized liposomes. Penetration of particles in majority of normal tissues is limited by continuous endothelial covering of vesicles [11]. Large particles and liposomes of more than 250 nm in diameter don't permeate practically through the capillaries even if intercellular contacts and pores in endothelium are sufficiently large. Close intercellular contacts (2-6 nm) between endothelial cells and the most normal tissues hinder extravasation even for very small liposomal particles (up to 30 nm in diameter). For example, tissues reach in sinuses and capillaries (liver, spleen, marrow) or pathological tissues are permeable for the particles up to 100 nm in diameter. A lot of primary and metastatic tumors have faltering and high permeable system of vesicles that is enough for exiting of small particles including liposomal ones. Thus vesicles physiology determines a heightened level of absorption in liver, spleen and tumor tissues. This fact explains the reasons of selective accumulation of liposomal cytostatic agents in tumor. Liposomes localization in tumor has been proved to be a result of a heightened level of penetration through the permeable tumor vesicles in combination with lymphatic drainage disorder [12, 13]. Specific structure of the blood vesicles and small size of liposomes cause stable accumulation of a liposomal medical agent into a tumor. This effect provides passive and directed delivery and increases pharmaceutical therapeutic effectiveness. The morphological researches of solid and ascitic tumors have shown that presence of PEG liposomes is limited mostly by tumor extracellular fluid; PEG liposomes release gradually a medical agent in a tumor area [9, 14].

It should be mentioned that liposomes could be used not only for directed delivery of the antitumor agents. Immune response becomes stronger in case of liposomal vaccines using. It happens because antigens associated with liposomes get directly the antigen-presenting cells. Liposomal hepatitis A vaccine was one of the first to develop [15]. There were incorporated proteins (influenza virus hemag-

glutinin) that help liposome and cell membranes fusion as well as antigen (viral capsid) into liposome. Nowadays these agents are often referred to as «virosomes». Virosome follows the natural way of viral particle and consequently an antigen fragment in combination with MHC II antigens are shown on the antigen-presenting cell surface, i.e. in a form wherein an antigen is recognized by T-helpers. Liposomes enable constructing the polyvalent vaccines, for example: several influenza strains vaccine, hepatitis A, hepatitis B and tetanus vaccines [16].

There are great possibilities in liposomes application in gene therapy [17, 18]. In genetic material delivery, liposomes act as protectors against nucleases and as compacting tool (positively charged liposomes) and as endocytosis initiator as well. It is known that the most important phase of virosome processing is fusion of its membrane and endosome membrane under action of hemagglutinin. In such a case liposome content gets to cytosol, in other words avoidance behavior of lysosomal enzymes takes place. Exactly this way is considered as primary one for liposomes carrying genetic material. Liposomes are considered as the most perspective carriers. Some compositions are found to be comparable by their transfection efficacy with the adenoviral vectors [6].

In many cases especially for gene therapy, it is important the address delivery into the cells of a required type. Immunoglobulines that have corresponding targets on the aim cells are often chosen as «molecular addresses».

By means of liposomal different proteins such as enzymes could be brought into an organism with the purpose of enzymotherapy and cytokines for the organism immune status correction. Very substantial investigations into creation of liposomes containing haemoglobin (haemosomes) with the purpose of receiving artificial blood substitutes have been carried out [5].

«Ideal» liposome model as a method of directed delivery of a medical agent into a cell is described in [6] (fig. 1). This liposome contains pharmaceutical substance, for example DNA in case of gene therapy, in its inner space. A molecular address and flexible polymer chains are immobilized on a liposome surface for decreasing absorption by RES cells. Fusion proteins are incorporated into a membrane. Moreover, a membrane consists not only of normal phospholipides making double layer (usually it is phosphatidylcholine) but of lipids that contribute to fusion with a cell

membrane (for example dioleinphosphatidylethanolamin) as well.

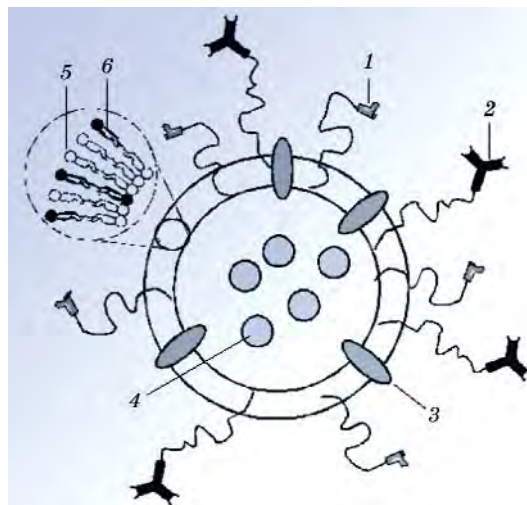


Fig. 1. «Ideal» liposome model [1]:

1 — polymer for steric protection from RES cells; 2 — «molecular address»; 3 — fusion proteins incorporated into membrane; 4 — pharmaceutical substance; 5 — double phospholipid layer; 6 — lipids that contribute to fusion with cell membrane

### Pegylated drugs

One way of increasing efficacy of protein structure medical products (interferons, hormones, growth factors, cytokines etc.) is chemical modification of their molecule addressed not to their structure change but to the native molecule transformation with polyethylenglycol (PEG). This chemical modification of peptide-structure pharmacological agents is purposefully directed to their tolerance improvement, immunogenicity lowering, half-life period prolongation [19].

PEG could be bonded with protein in several positions, but some of them are premium, for example, most often covalent connection with protein (interferon  $\alpha$ -2b) takes place at the lysine  $\epsilon$ -amino group nitrogen atom or at the histidine imidasole group. These bonds allow to activate PEG hydroxyl groups and to build molecule, that has covalent bonds on the target places. Moreover, PEG straight covalent bonds at lysine and arginine positions (carboxyl ends) directly prevent modified molecules from trypsin breakdown [20].

One of the most important resources of pegylated molecules is high hydrophilicity, that controls new characteristics of modified peptide. High content of hydrogen atoms even in one PEG molecule enables it to bond with 2–3 water molecules. This effect leads to formation

of a «water cloud» around a modified molecule and therefore its hydrodynamic radius raises.

It is some kind of a water «shield» around modified molecule that raises solubility and bioavailability of a medical agent and protects molecule from other proteins (neutralizing antibodies, complement). Thus pegylated peptides are noticeably more protected from opsonization, active phagocytosis and endocytosis of the cell structures. Monomethoxyethylenglycol is used the most often for bonding peptide molecules. Its molecule has one hydroxyl group that conjugates with protein; other ends of PEG molecule contain non-reactive methyl groups [20, 21].

Changes in pharmacokinetic and pharmacodynamic characteristics of pegylated peptides depend on PEG molecule weight and on specific bond places. For example, direct correlation between PEG molecule weight and peptide half-life period was shown: increase of this index ranges from 3–5 times (uricase, streptokinase) to approximately 500 times (superoxide dismutase) [22, 23]. One more important factor, that affects the pharmacokinetic and pharmacodynamics of PEG-modified peptides is PEG chains structure: branched PEG molecule forms deceleration of medical agent active transport. It also causes prolongation of active circulation of medical agent. Less immunogenicity of the modified medical agents while saving their main pharmacological characteristics is also resulted from PEG chains branched structure. Similar effects could be obtained by other means — by peptide conjugation with several PEG molecules having linear chains structure [20–22]. In this case, interleukin-2 (IL-2) is a well-known example. IL-2 molecule is very small, that's why it is easily filtrated by kidneys and has a very short half-life period. Junction of IL-2 and PEG with  $M_r > 20$  kD doesn't interfere practically in protein pharmacodynamics but increase in PEG molecular weight to 60–70 kD noticeably slows down conjugate filtration and increases its half-life period and bioavailability [24].

Modern technologies of pegylation of biologically potential peptide molecules resulted in greatly extension field of their practical application at present and in the future. Since the advent of PEG-modified peptides forming the number of pharmacodynamic and pharmacokinetic effects has been allowed which were impossible before now for the specific peptide. Results of the clinical trials with pegylated erythrocytes,  $\alpha$ -2b interferon, adenosine dea-

menase, and tumor necrosis factor soluble receptor are brilliant examples of this phenomenon [25–28].

**Pegylated erythrocytes.** The most actual problem is blood erythrocytes transfusion to the chronic patients, who receive transfusions regularly. They are the patients who suffer from sickle cell disease, thalassemia, and the patients being treated with hemodialysis because of chronic renal insufficiency. It is clear that this group of the patients is mostly inclined to external antigen aggression and to a risk of alloimmunization forming [19]. Creation and clinical trials of PEG-modified erythrocytes were performed to solve this problem. Physicochemical structure of PEG, together with its viscosity characteristics, high molecule stability and hydrodynamic parameters enabled to create high-performance conjugate with erythrocytes. During the experiment, the short linear PEG chains made it possible to «screen» Rh factor and other surface antigens, while the long chains promoted blocking of erythrocytes adhesion between each other and that of the vesicles endothelium. Furthermore the long PEG chains prevented erythrocytes from damage and their deformation in a vascular bed. Branched PEG chains with compact areactogenic zones blocked external antigens connection and their production effectiveness regarding to erythrocytes injected from the outside. PEG erythrocytes obtained in such a way showed their high performance both on the animal models and in the researches in humans. Besides there were no side effects that usually accompany hemotransfusions. In such a case total hemodynamics was certainly improved, oxygen transport cell function was prolonged and indices of rheology parameters were substantially improved. It should be noticed that these investigations made a basis for creation of new pegylated blood cells that would find practical application especially in «transplant against host» reactions [27].

**Pegylated  $\alpha$ -2b interferon.** One more field of future use of peptide structure pegylated agents is antiviral therapy. Traditional regime of  $\alpha$ -interferon administration is 3 million IE 3 times a week. It is important to note that peak interferon concentration is observed within 8–12 hours after hypodermic injection; its half-life period is 6 hours at average. It is clear that together with stable concentration periods there are periods when level of interferon in serum and biological tissues could come down to the almost indeterminable values. It is logically to assume that for reaching

the acceptable therapeutic level of native exogenous interferon (IFN), it is necessary to change its pharmacodynamic and pharmacokinetic parameters fundamentally. This requirement caused creation of basically new pharmaceutical form of  $\alpha$ -2b interferon in conjugation with PEG. Pegylated IFN has noticeably better biological profile than ordinary interferon; it appears in greatly increased half-life period of pegylated analog and in decreased immunogenic characteristics. Coupling of a relatively small PEG molecule of molecular weight 12 kD to interferon *in vivo* showed that maximal protein concentration is reached in 15–44 hours and remains during 48–72 hours. Therefore effective half-life period makes up 40 hours in average. Slowing down of PEG interferon clearance from plasma provides its circulation in blood during a week. Pharmacokinetic and pharmacodynamic parameters provide exactly really high effectiveness of  $\alpha$ -2b PEG interferon in comparison with a standard analog. Besides, PEG molecular weight provides not only liver but kidney clearance as well. One more important advantage of pegylated  $\alpha$ -2b interferon in contrast with normal recombinant interferon is ability to use it for cirrhosis treatment because this group patients was usually deprived of full antiviral therapy. So, the balance between antiviral activity and long period of partial ejection is typical for pegylated  $\alpha$ -2b interferon; it allows to prescribe an agent one time a week and to remove PEG metabolites from an organism effectively [19, 23, 28].

### Receptor-dependent endocytosis

Depending on cell absorption mechanism of the macromolecules and particles, endocytosis could be divided into constitutive (or liquid-phase) and receptor-dependent one. In the first case a nonselective process takes place during which concentration of the substances that are absorbed as a part of vesicles corresponds to a concentration of the substances in the extracellular liquid. Receptor-dependent endocytosis (figure 2) is exclusively selective concentrating mechanism that enabled the cells to absorb lots of specific ligands without absorbing large volume of extracellular liquid [29–32]. At the same time macromolecules that have limited number of binding sites on the plasmalemma are absorbed. These sites have high affinity to the certain substances. The sites absorb separately substances from the cell space and concentrate them. In addition the liquids and dissolved strange mole-

cules that don't bind with the receptors are slightly absorbed. Thus effective flux of the specific molecules into a cell is provided. During endocytosis vesicles are formed in the places of plasmalemma invagination; on the part of cytoplasm plasmalemma is coated with fibrous material — membranous protein clathrin. The pits coated with clathrin that could occupy up to 2% of some cells surface are formed in advance. By means of these coated pits where the corresponding receptors are located a number of substances, such as low-density lipoproteins, growth factors, hormones etc. are internalized [1].

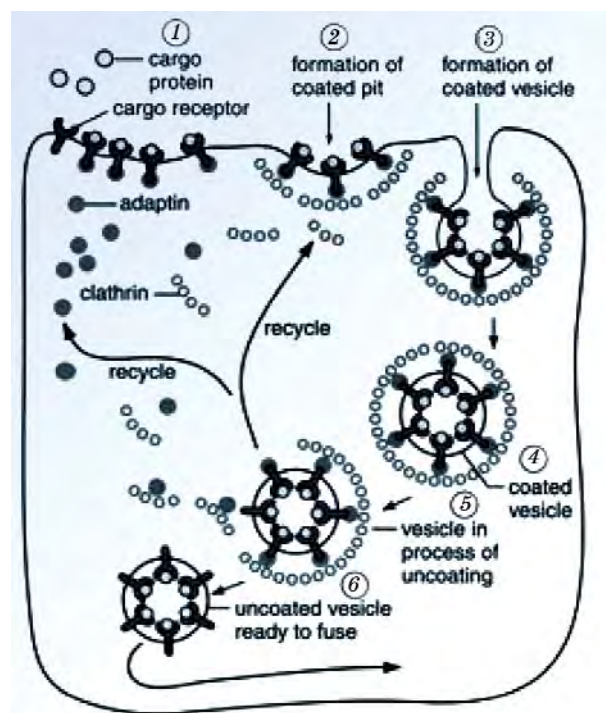


Fig. 2. Receptor-dependent endocytosis phases: Ligand and receptor binding (1), coated pit forming (2), Clathrin vesicle forming (3), intracellular utilization (4–6)

**Use of protein vectors that are specific to cell receptors.** In developing of common use anticancer agents, hybrid covalent bound conjugates of «protein vector — chemoagent» type method was widely used. Selectivity of conjugates action is obtained due to presence of the specific receptors on the tumor cells surface that are recognized by cargo protein or antibody or due to a considerably higher expression level of cargo protein receptors on the tumor cells surface in comparison with the normal cells. The data concerning successful use of cytotoxic conjugates created on a basis of cytotoxic antibiotics and cargo molecules

delivering antibiotic directly into tumor cells or tumor vessels endothelial cells are published in a number of works [33]. Oncofetal proteins, transferrin, monoclonal antibodies to specific tumor antigens, hormone-like peptides etc. are widely used as cargo molecules.

Conjugation of cargo protein and agent could be carried out in several ways: by means of chemical cross-linking (in a simple case it is disulfide or thioester bridges), polyethylene glycol or polypeptide linker, avidin-biotin technology etc. Methods of bioconjugation are described more detail in the work [34]. In any case method of conjugation should satisfy two main criterions: high reaction yield and ability of intracellular breakdown. The last requirement is unessential. In such a case vector «independence» of an agent is determined by sizeable length of a linker. For these linkers creation usually PEG with molecular weight up to 2–3 kD is used [35].

The cytotoxic antibiotics and apoptosis inductors etc. are used as antitumor agents.

It is clear that specificity of conjugate action is determined first of all by a structure and type of cargo protein.

**Transferrin-transferrin receptor.** It is well known that ferrum ( $\text{Fe}^{3+}$ ) transport in an organism takes place as a complex with globulin protein under transferrin (Trf). Ferrum transport into a cell is a result of endocytosis due to Trf interaction and its receptor (TRFR) [36, 37]. Transferrin is widely used as a cargo protein for directed delivery of the anticancer agents, proteins and genes to a tumor cell for which higher TRFR expression level is typical. Tfr-specific intracellular delivery is achieved by conjugation of this cargo protein with some antitumor agents (doxorubicin, daunorubicin) and protein toxins (CRM107, ricin) [38]. Use of such constructions allows to decrease essentially toxicity of chemotherapy and to withstand somehow cancer cells acquired resistance mechanisms.

**Hormones and their receptors.** Peptide hormones having specific receptors on their cells surface also could be effectively used as cargo molecules. Particularly it was determined that variant carcinoma and prostate overexpression in the tumor cells was observed for decapeptide gonadoliberin receptors at carcinogenesis of breast, ovarian carcinoma and prostate [39, 40]. That's why gonadoliberin could be used for directed delivery of medical agents to such kinds of malignant tumors. Thus for conjugate consisting of gonadoliberin, PEG and camptothecin high antitumor activity on the mouses was shown. This con-

struction was non-toxic and at the same time increased gonadoliberin concentration showed lack of considerable physiological effect on reproductive functions of the tested animals [39].

Insulin receptors, found almost in all organism tissues, are of the utmost interest for studying [41]. In particular, a lot of tumor cells are characterized by a high level of insuline receptors expression [42–45].

In a structure of every hormone the centers that determine interaction of a hormone only with the target cells could be marked [46]. Consequently, it seems possible to create a liposomal vector based on a structure of that site of insuline molecule that is charged for interaction with a receptor. It is known from the publications that an area on the C-terminal ends of A- and B-chains of an insuline molecule is responsible for binding and biological activity manifestation [47].

In [48] it is described synthetic decapeptide which aminoacid sequence is correlated with 19–26 aminoacid residues of B-chain and 20–21 aminoacid residues of A-chain insuline molecule bonded against each other with a short peptide. A short fragment which aminoacid sequence corresponds to 23–26 aminoacid residues of B-chain and 20–21 aminoacid residues of A-chain insuline molecule interconnected with a short peptide bond has been created as well [48]. This fragment includes a hydrophobic sector of C-terminus of insuline molecule B-chain. This is the most important sector that is responsible for combining with a receptor and for dimeres formation [49]. Both peptides were acylated on the N-terminus by ether of palmitic acid. After they were built in the lipid double layer. Acylated peptides included into the liposomes could firmly bond with insuline receptor of rat pheochromocytoma PC12 cells *in vitro*; further it resulted in receptor-dependent liposomes endocytosis [48].

**Oncofetal proteins.** Some oncofetal proteins, for example  $\alpha$ -fetoprotein (AFP), could also be used as cargo molecules for the medical agents delivery into the tumor cells. Advantages of their use are absence of immunogenic characteristics, high affinity to the receptors and high level of receptors expression on the tumor cells [50, 51]. It was shown that AFP receptors were expressed on a surface of the overwhelming majority of the tumor cells while on the normal cells the receptors weren't expressed or expressed in the minor amounts [52].

The abovementioned experimental data concerning an expression level of the AFP

receptors and high endocytosis speed enabled to make an assumption concerning high selectivity of delivery of conjugates of AFP with cytotoxic agents into the tumor cells.

Conjugates of AFP with different chemotherapy agents (phthalocyanines, chlorines, alkaloides and anthracyclines) were created and studied. Research results have shown that use of AFP as a cargo molecule made it possible to increase cytotoxicity of the most medical agents studied concerning tumor cells lines [52–59]. Conjugates of AFP with doxorubicine were characterized by high antitumor activity and their cytotoxic activity concerning antibiotic resistant tumor cells was in large excess over uncombined doxorubicine activity [52–54]. Investigations on the mouse solid tumors models have shown that AFP conjugates with cytotoxic agents are upon pronounced inhibitory action on the tumors and in comparison with uncombined antibiotics they prolong notably life time of the experimental animals [52, 53]. Positive results were received as well when conjugates of epidermal growth factor (EGF) and receptor-bonding EGF fragment were used with the antitumor agents [56–63].

Another possible approach is creation of the directed action agents in a form of AFP conjugates with antisense oligonucleotides (ASON) to mRNA genes that play a key role in regulation of cell proliferation and apoptosis. ASON using experiments for inhibition of mRNA genes translation which hyperexpression leads to transformation of normal cells to tumor showed high ASON specificity to their targets [64, 65]. Unlike most chemotherapy agents, ASON are easily biodegraded and egested from an organism. To avoid their degradation by endonucleases and exonucleases, modified oligonucleotides are used whereof the most perspective are phosphorothioate derivatives [2]. At the same time there is a number of problems due to insufficient effectivity of their delivering into the tumor cells.

**Monoclonal antibodies and immunoliposomes.** Monoclonal antibodies (mAb) to different receptors on the cancer cells surface (TRFR, epithelium growth factor receptor, CD-receptors) are the most wide-used cargo molecules. Production of such mAbs underlies an oncovaccine action. Moreover, the radiolabeled mAbs that are bond with a cell and cause its death because of presence of radionuclides in their structure are sometimes used. Besides mAbs are often conjugated with cytotoxic antibiotics and other bioactivity substances by different linkers (avidin- biotin, PEG, etc.)

[56]. Use of conjugates based on mAb to receptors is often more effective than use of natural ligands as cargo molecules. For example, Tfr is characterized by limited ability to permeate through hematoencephalic barrier that doesn't make it possible to use Tfr in therapy of brain oncological diseases. At the same time antibodies to TRFR permeate freely to different brain tissues [67].

An original tendency in creation of directed drug delivery systems is immunoliposomes — liposomes with attached monoclonal antibodies [8]. Monoclonal antibodies provide specific binding of liposomes with antigen-positive cells while liposomes carry appropriate hydrophobic or hydrophilic chemotherapy agent.

Nowadays there are three types of immunoliposomes: A, B and C [68]. mAbs of A type immunoliposomes are covalently bound with normal liposomes by a short linker. B type is already PEG liposomes that is bound covalently with mAb by a short linker. Type C (Pendant-type PEG-immunoliposomes) is steric stabilized PEG-liposomes with mAb attached to distal terminal PEG end.

By means of type A liposomes it was shown that immunoliposomes were more effective in delivering drugs to the target cells in comparison with normal liposomes both *in vitro* and *in vivo* tests [69]. However immunoliposomes binding with the target cells *in vivo* was more complicated. Study of immunoliposomes *in vivo* showed that antibodies attaching to liposomes strengthened their absorption by RES mononuclear leukocytes. Effectivity of liposomes adhesion with the target cells depended on antibodies thickness on the liposomes surface. Immunoliposomes absorption by RES cells and endothelial barrier compelled the scientists to create a new type of liposomes. It resulted in construction of steric stabilized immunoliposomes with a prolonged period of circulation in blood.

In the earliest works concerning long-persistent immunoliposomes creation, the antibodies were bound by short hydrophilic linker close to a surface of liposomes (type B); these steric stabilized liposomes contained phospholipids with PEG modified main groups [70]. The liposomes kept ability of long-continued circulation but interaction with the target cells was suppressed by PEG blockade [71].

Later mAb were attached to the distal ends of PEG chains bound with type C liposomes. It resulted in keeping the steric stabilized liposomes ability to bind specifically with a target cells surface and to be protected from absorption by RES mononuclears [72].

Nowadays for the purpose of getting the stable connection of antibodies with PEG, three conjugation methods are used for covalent binding mAb and PEG terminal ends: by thioether bond [73], by amid groups [68] and by hydrazones [74].

It should be mentioned that there are some requirements to antibodies used in immunoliposomes construction. They should keep their specificity when conjugate with liposomes, have enough affinity for low concentration liposomes binding and have low immunogenicity. For this purpose chimeric and humanized mAb and Fab antibodies fragments are used. Antibodies should be intensively internalized by the target cells through endocytosis, have biological activity and intensify antitumor response. Monoclonal antibodies should be manufacturable and have enough storage life [74].

There are some requirements to antigens being a target for liposomes. It should be strong and homogeneous at express in tumor tissue and not disappear from a cell surface. Antigen desquamation from a tumor cell surface should be minimal to avoid immunoliposomes and soluble antigen binding or clearance intensification. «Antigen-immunoliposome» complex should pinocytate into a tumor cell. Bond between antigens and liposomes should be stable in blood. Linker shouldn't bind with a molecule site recognized by antigen, should be interimmunogenic, atoxic, avoid opsonization, be inert towards a medical agent inside liposome and liposome membrane stability and not put steric obstacles.

Ratio of antibodies and lipids in immunoliposome is an important factor [68]. Thus, at 1:50 weight proportion one liposome was added to 24 mAb molecules while at 1:1 proportion — 935 antibodies were. Specific accumulation of immunoliposomes with 1:50 proportion was 3% of a given dose, and accumulation of immunoliposomes with proportion 1:1 was 60% of a given dose. Immunoliposomes absorption by the liver cells was reduced from 50% of a given dose for the liposomes with low content of antibodies to 12% for the liposomes with high content of antibodies. At that, absorption of liposomes containing small amount of antibodies didn't differ from normal liposomes absorption. Liposomes that didn't bind with the target cells at the several first passages through tumor capillaries were accumulated in liver and spleen [68].

Specific delivery of antitumor agents by means of immunoliposomes stimulated better therapeutical effectiveness and reduce toxic-

ity as compared with normal liposomes [75]. It was clearly demonstrated on the mouse solid tumor models [76] and on the nude mice heterologous grafts of human B-cell [73].

By now several immunoliposome preparations perspective for use in oncological practice has been described. They are directed against the cells that express the following antigens: CD71 (transferrin receptor), Her2/neu (epidermal growth factor receptor) [77], HLA-DR (MHC II antigens) [78, 79], CD19 (B-cells marker) [73], LL2 (B-cells lymphoma antigen) [80].

**Cell penetrating peptides.** Until quite recently use of polypeptides and oligonucleotides for research and therapeutic purposes was restricted because of their low permeability through biomembranes and relatively quick degradation inside a cell. This restriction was an obstacle for biomedical researches and also for pharmaceutical industry. Hydrophilic macromolecules transport into cytoplasm and nucleus bioplast seemed to be impossible without membranes destruction. At the same time delivery of biologically active macromolecules inside a cell open wide prospects for the biological objects manipulation. That's why discovery of peptides that are able to permeate into a cell without membrane proteins assistance and put intracellular transport in force of protein fragments and oligonucleotides bound with them opens a new phase in development of biology and medicine. Such peptides are called «cell penetrating peptide» (CPP). Sometime such type of peptides is called «Troic horse» as well [81-83].

CPP is isolated from proteins of different organisms from viruses (HIV-1, herpes, influenza) up to vertebrates (cayman). Typical and most studied CPP is penetratine (pANTP). It is 16 aminoacids peptide isolated from Antennapedia Drosophila melanogaster protein [81]. TAT peptide is a fragment of immunodeficiency virus HIV-1 capsid protein [82, 83], and VP22 peptide is a fragment of herpes simplex virus capsid protein [84].

According to physical-chemical characteristics CPP could be divided into two groups: hydrophobic (FGF of Kaposi's sarcoma glycoprotein, gp41 of HIV-1 glycoprotein and Ig(v) of cayman immunoglobulin light chain) and amphiphile (Hel 11-7 of influenza virus hemagglutinin, TAT, VP22 and pANTP). Length of such peptides varies from 11 to 30 aminoacids. Analysis of CPP aminoacid sequence didn't find homology between them, but it was noticed that almost always there are several arginine molecules. Investigation of



such regularity enabled some researchers to consider internalization as a property of arginine-rich peptides. However later on amino acid sequences that don't contain arginine and are in a position to permeate through cytoplasm membranes have been synthesized [85].

Mechanism of CPP transport through a cell membrane is unknown at the present. But it is known that transport takes place without membrane proteins assistance, it practically doesn't depend on carbohydrates expression character and is energy independent [86]. Some cell penetrating peptides (TAT, VP22) are able to permeate through intracellular membranes and accumulate into the cell nucleus. It is experimentally proved that CPP penetrate in different cell types with equal effectiveness and even could overcome a mammal's histohematogenous barrier [87].

It was shown that these peptides could transport amino acid and oligonucleotide sequences covalently bound with them through a cell membrane. These sequences have molecular weight up to several kD and resist intracellular hydrolysis for a long time because they are located out of lysosomal enzymes effective area. Thus, cell penetrating peptides could act as transport mechanism of physiologically active macromolecules parts

into a cytoplasm or even into a cell nucleus [88]. Such unique CPP characteristics enabled to create directly the chimeric molecules consisting of cell penetrating peptide and a macromolecule fragment covalently bounded with it [87, 88].

Modern literature review concerning approaches to creation of the purposeful drug delivery systems of anticarcinogenic vaccine preparations and genotherapy agents are made in the article. Targeted drugs delivery is implemented by use of liposomal forms and receptor-dependent endocytosis. The main problem of liposomal preparations is bad pharmacokinetic indices conditioned by liposomes instability, their quick opsinization at intravenous administration and further absorption by RES cells. This problem is mainly solved by means of covalent binding with polyethylene glycol that gives hydrophile characteristics to liposomes. Receptor-dependent endocytosis is used for specific drug delivery to the target cells. The transferrin, hormones (for example gonadoliberin and insulin), oncofetal proteins (such as  $\alpha$ -fetoprotein and epidermal growth factor), monoclonal antibodies and peptides able to permeate inside the cell are used as protein vectors.

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**СИСТЕМИ ЦІЛЕСПРЯМОВАНОГО  
ДОСТАВЛЕННЯ ЛІКАРСЬКИХ ЗАСОБІВ.  
БІОТЕХНОЛОГІЧНІ АСПЕКТИ**

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В огляді узагальнено дані літератури щодо підходів до створення систем цілеспрямованого доставлення лікарських засобів, які базуються на використанні ліпосомальних форм та рецепторопосередкованого ендоцитозу. Розглянуто підходи до використання систем спрямованого доставлення при створенні антиканцерогенних, антибактеріальних препаратів, вакцин, а також у генотерапії.

**Ключові слова:** системи цілеспрямованого доставлення, лікарські засоби, ліпосоми, ендоцитоз, протираккові препарати, моноклональні антитіла.

**СИСТЕМЫ ЦЕЛЕНАПРАВЛЕННОЙ  
ДОСТАВКИ ЛЕКАРСТВЕННЫХ СРЕДСТВ.  
БИОТЕХНОЛОГИЧЕСКИЕ АСПЕКТЫ**

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В обзоре обобщены данные литературы относительно подходов к созданию систем целенаправленной доставки лекарственных средств, основанных на использовании липосомальных форм и рецепторопосредованного эндоцитоза. Рассмотрены подходы к использованию систем направленной доставки при создании антиканцерогенных, антибактериальных препаратов, вакцин, а также в генотерапии.

**Ключевые слова:** системы целенаправленной доставки, лекарственные средства, липосоми, эндоцитоз, противораковые препараты, моноклональные антитела.