Objectives. Over the last few years, medicinal chemistry research has been focusing on the creation of molecules that can target particular body systems, organs and tissues, thus abating systemic toxicity and side effects, and, most of all, boosting therapeutic potential. This goal can be achieved through the specific interaction of such drugs with active sites of cellular receptors. For example, glycoprotein receptors that can be found on cellular surfaces in neural tissues and liver parenchyma, selectively bind various glycoproteins and glycosides, facilitating their penetration into cells. This review describes how certain parameters of ligand structure (the nature and length of the spacer between carbohydrate and non-carbohydrate fragments of the molecule, number of carbohydrate residues per molecule, etc.) influence the penetration efficiency of synthetic glycoconjugates into liver cells.

Methods. This review article summarizes 75 research papers and discusses data from in vitro and in vivo experiments showing which structures of synthetic carbohydrate derivatives are optimal for targeted drug delivery into liver cells.

Results. The surface of liver cells (hepatocytes) contains a significant number of asialoglycoprotein receptors (ASGP-R) that are almost never found elsewhere. This makes ASGP-R an ideal target for the directed treatment of liver diseases, including such difficult, socially important conditions as hepatocellular carcinoma and Hepatitis C. A number of various ligands and targeted (to ASGP-R) delivery systems have been designed. Such molecules always contain derivatives of mono- and disaccharides, most commonly D-glucose, D-galactose, D-lactose and N-acetylglucosamines. This review contains the chemical structures of carbohydrate-based ligands.

Conclusions. Glycolipids based on D-carbohydrates, when in liposomes, facilitate penetration into liver cells by a receptor-mediated, clathrin-dependent endocytosis mechanism that is activated upon contact of the carbohydrate-containing ligand fragment with the active site of ASGP-R. It can be addressed by the use of monovalent derivatives of carbohydrates as well as polyvalent glycoconjugates. Alterations in the ligand structure and the number of liposomal modifications can boost the therapeutic effect. The distance between the liposomal surface and the carbohydrate residue (spacer length), as well as the hydrophilic-lipophilic balance of the ligand molecule, have a great effect on the affinity and cellular response.

Ключевые слова: glycoconjugates, asialoglycoprotein receptor, receptor-mediated endocytosis, targeted delivery, liver cells.

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Структурные особенности синтетических гликоконъюгатов и эффективность их взаимодействия с гликопротеиновыми рецепторами на поверхности гепатоцитов

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Цели. Последние несколько лет исследования в области медицинской химии уделяют большое внимание созданию молекул, направленно воздействующих на конкретные системы организма человека, органы и ткани, что помогает снизить общее токсическое воздействие препаратов на их основе, уменьшить степень проявления побочных эффектов, а самое главное – многократно усилить их терапевтический эффект. Это может быть достигнуто при помощи специфического взаимодействия подобных веществ с активными центрами клеточных рецепторов. Например, класс гликопротеиновых рецепторов, располагающихся на поверхности клеток нервной ткани и паренхимы печени, селективно связывает различные гликопротеины и гликозиды, способствуя их проникновению внутрь клеток. В обзоре рассмотрено влияние таких особенностей структуры лигандов, как природа и длина связующего звена (спейсера) между углеводной и неуглеводной частями молекулы, количество углеводных остатков в составе одной молекулы, а также ряда других, на эффективность проникновения синтетических гликоконъюгатов в клетки печени.

Методы. В обзоре проанализировано 75 публикаций и обобщены результаты исследований, в которых с помощью in vitro и in vivo экспериментов устанавливается, какая структура искусственно синтезированных производных углеводов окажется наиболее оптимальной для направленной доставки лекарственных средств в клетки печени.

Результаты. На поверхности гепатоцитов (клеток печени) в большом количестве представлен асиалогликопротеиновый рецептор (ASGP-R), который почти не встречается на других типах клеток, что делает его идеальным рецептором-мишенью для направленного лечения заболеваний печени, в том числе таких трудноизлечимых социально значимых заболеваний, как гепатоцеллюлярная карцинома и гепатит С. Разработан ряд разнообразных лигандов и систем направленной доставки к ASGP-R. Такие молекулы обязательно имеют в составе производные моно- и дисахаридов, чаще всего применяются D-глюкоза, D-галактоза, D-лактоза и N-ацетилглюкозамины. В обзоре приводятся примеры химических структур углеводсодержащих лигандов.

Заключение. Гликолипиды на основе D-углеводов в составе липосом обеспечивают их проникновение в клетки печени по механизму рецептор-опосредованного клатрин-зависимого эндоцитоза, который активируется при контакте углеводсодержащей части лиганда с активным центром ASGP-R. Показано, что для этого можно использовать как моновалентные производные углеводов, так и поливалентные гликоконъюгаты. Варьируя структуру лиганда и количество добавляемых к липосоме модификаций, можно достичь наибольшего терапевтического эффекта. Большое влияние на аффинность и клеточный ответ оказывают расстояние от поверхности липосомы до углеводного остатка (длина спейсера) и гидрофильно-липофильный баланс молекулы лиганда.

Keywords: гликоконъюгаты, асиалогликопротеиновый рецептор, рецептор-опосредованный эндоцитоз, направленная доставка, клетки печени.

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Novel pharmaceutical approaches in the design and development of medications are expected to lead to a rise in revolutionary drugs with high bioavailability, biocompatibility and efficacy, and low toxicity. One of the ways to solve the existing difficulties in drug design is the creation of nanoparticles that carry low yet efficient doses of medication. The great variety of nanosized delivery systems allows us to design therapeutic complexes with the required characteristics. A number of in vitro and in vivo studies have shown that liposomes (lipid vesicles with a two-layer membrane) possess all the necessary features to deliver any type of medication.

The liver’s role in the metabolism of toxic substances implies that its cells (hepatocytes) are often affected by drugs, microbes and toxic molecules, which may lead to a number of liver diseases. Such diseases are the fifth most common cause of death. Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world, and the third most deadly type of cancer [1, 2]. HCC development is driven by two major types of the hepatitis virus, Hepatitis B and Hepatitis C (HBV and HCV, respectively). Approximately 2 billion people across the world are infected by HBV and 320 000 cases are fatal every year [3], whereas 170 million people in the world are infected by HCV [4, 5]. Since HCC and other liver diseases, such as fibrosis and cirrhosis, mainly affect hepatocytes [6], the targeted delivery of therapeutics directly to these cells seems to be a most logical approach. In order to develop a hepatocyte-targeted delivery system, an asialoglycoprotein receptor (ASGP-R) [7] was selected as a target receptor. It is often found on the surface of hepatocytes but not as much on the membranes of other cells.

Today, research is focusing on the establishment of optimal glycolipid structures that will give liposomes “targeting” features. Several systems of gene delivery that are based on such ligands have shown exciting clinical results, attracting the attention of the scientific community and demonstrating the prospects of nanotherapeutics.

**Structure and functions of the asialoglycoprotein receptor ASGP-R**

ASGP-R, also known as the “Ashwell–Morell receptor,” was the first mammalian cellular lectin discovered in the 1960s, during studies on the metabolism of plasma glycoproteins [8, 9]. The main function of this receptor is binding to cellular fibronectin, prothrombin components, liver lipoproteins and immunoglobulin A (IgA). The role of ASGP-R is to mediate the homeostasis of serum glycoproteins by balancing between binding to and the endocytosis of a wide range of glycoproteins that bear galactose residues or N-acetylgalactosamine at the termini [10]. These glycoproteins undergo endocytosis through clathrin-presenting areas, after which they are transported into lysosomes for acidic degradation. ASGP-R is widely present on the surface of parenchymal liver cells, making up to (1–5)×10⁵ of binding sites per cell [11]. Apart from this, the interaction of the receptor with cellular components of pathogens is a major reason for the generalization of certain liver diseases, particularly those caused by hepatitis viruses A and B, as well as the Marburg virus [12–15].

Mammalian ASGP-R consists of two homologous polypeptide subunits, main and auxiliary that are encoded by two genes [13, 16]. In humans, the main subunit H1 and the auxiliary subunit H2 are 46 and 50 kDa in size, respectively. Each subunit is a transmembrane C-type protein, with a short N-terminus in the cytoplasm; an internal part that runs throughout the membrane; and a C-terminus with a Ca²⁺-dependent domain responsible for carbohydrate recognition, on the outer side of the membrane [17]. Combinations of different subunit ratios lead to functional homo- and heterooligomers with different receptor configurations. It has been established that the most common configuration is the conjugate of two H1 subunits and one H2 subunit (Fig. 1). This conjugate shows the highest affinity to the ASOR ligand (asialo-orosomucoid) that binds to ASGP-R, similarly to lactoferrin – serum glycoprotein [11, 18].

**Fig. 1. Schematic depiction of ASGP-R that showing a heterooligomer made up of two H1 subunits and one H2 subunit. The figure shows the spatial arrangement of the receptor’s binding sites [19].**
The carbohydrate recognition domain (CRD) in ASGP-R subunits belongs to the C-type family (Ca\(^{2+}\)-dependent) [20]. The majority of CRD C-type domains selectively bind to D-mannose, D-glucose and their derivatives (Man-type ligands), or D-galactose and its derivatives (Gal-type ligands). The binding of D-galactose to the receptor (Fig. 2) occurs in the presence of Ca\(^{2+}\) ions under basic conditions [21–23].

Fig. 2. Binding of the N-acetylgalactosamine (GalNAc) molecule to the recognition domain of ASGP-R.

Between hydroxyl groups at 3 and 4 carbon atoms in the pyranose ring of GalNAc and \(^{187}\)Asp, \(^{198}\)Glu, \(^{185}\)Gln, \(^{211}\)Asp and \(^{210}\)Asn amino acid residues, the interaction is due to hydrogen as well as coordination bonds (with the participation of the Ca\(^{2+}\) ion). The hydrogen atom in the amide bond and the nonpolar GalNAc region between 3 and 6 carbon atoms participate in the creation of hydrophobic interactions with \(^{202}\)His and \(^{189}\)Trp amino acid residues, respectively [19].

ASGP-R facilitates clathrin-mediated endocytosis [24]. This mechanism is used upon interaction with a transmembrane receptor that activates a signaling cascade and lets the particles through. Clathrin domains occupy only 0.5–2% of the total cell surface area, meaning that substance transport by a clathrin-mediated mechanism is quite selective. For successful recognition, liposomes and other substances should be first labelled by apolipoproteins that are commonly found in plasma [25]. After entering the cell, this aggregate is found inside an early endosome whose membrane later fuses with the outer layer of the liposome, maturing to form a late endosome. Depending on the structure of the parental liposome, its charge and presence/absence of specific ligands on the surface, the late endosome can either become a lysosome (upon enzymatic action) leading to the degradation of the whole complex, or its contents can be released into the cytoplasm where they can affect the organoids [26, 27]. Research on the mechanisms of endocytosis and ways of blocking it has shown that penetration of the cells does not occur at 4 °C, but the process of molecule recognition by receptors remains the same [28]. This is why it is possible to determine whether the transport is receptor-dependent and selective while using low temperatures for studying mechanisms of penetration for certain liposome structures. It has also been demonstrated that the human Hepatitis C virus enters hepatocytes via this mechanism [29].

ASGP-R has also been found on the surface of hepatocytes in other mammals, including rabbits [30], mice [31], and rats [32], although the size and number of subunits differ slightly between species. Despite the differences in receptor structure in various mammals, the amino acid sequence is rather conservative and, potentially, originates from the same common gene. For example, the H1 subunit is 80% identical to the rat lectin-1 (RHL1), and the H2 subunit is 62% identical to the RHL2 [33]. This fact allows us to project the in vivo experimental data on to the expected results of clinical studies.

### Principles of targeted drug delivery to liver cells

It is known that in chemotherapy more than 90% of molecules of cytotoxic agents are captured by healthy tissues and only 2–5% reach tumors [34]. This is why it is so important to create drug delivery systems that could be selective and would only reach target organs [35, 36]. Receptor-mediated endocytosis is a very promising approach for targeted drug delivery because it allows high drug concentrations in target cells to be reached, thus boosting efficacy and decreasing side effects.

Optimizing the ligand–receptor interaction has demonstrated that affinity is affected by a number of properties of the carbohydrate ligand. For example, it is known that the recognition domain binds cyclic D-galactose derivatives and acyclic D-galactosides equally well. The most efficient interaction with the receptor is observed for derivatives of D-galactose and D-glucose; lactose and D-mannose are less effective for hepatocytes, but they have a higher affinity for the receptors of Kupffer cells [37]. Affinity of D-galactose increases by 100–1000 times when the number of carbohydrate residues at the terminus of one ligand molecule grows from 1 to 3–4, due to the cluster effect [38]. To this end, monovalent and polyvalent ligands can be used to target ASGP-R.

The distance between the carbohydrate residue and the liposome surface is a crucial parameter for interaction with lectins [39, 40]. Construct design should take into account the minimal distance between
Theoretical calculations and experimental data have confirmed that a long spacer makes the carbohydrate residue more mobile (glycolipid 1c), providing easier access to the binding site of the receptor protein. For example, D-galactosides with a 20 Å long spacer bind to the receptor even at low concentrations, whereas a 4 Å long spacer requires more active molecules [45]. Similar results have been obtained in experimental attempts to lower serum cholesterol: the effective dose of glycosides with a 20 Å long spacer is 30 times lower than that for compounds with a 4 Å long spacer [46]. For the spontaneous binding of carbohydrate residue to the receptor, the spacer length should be 25–30 Å on average, and if the carbohydrate residue is cyclic, a slightly longer distance to the nanoparticle surface is required [47]. At the same time, there are successful examples of shorter spacers, which are 15 Å [38], 11.05 Å [48], and 10.1 Å long [49]. It seems that there is no universal, ideal spacer length for all types of carbohydrate ligands, and this length depends on the spacer’s nature and the type of carbohydrate residue. Studies on transfection activity of lipoplexes, which are based on aliphatic glycosides that contain a quaternary nitrogen atom, have shown that the cyclic form requires a spacer that is 6 methylene units long (2a–e), and the acyclic form requires only 2 methylene units (3a–e) [50]. It has also been shown that pyranosides, which have a glycosidic bond at the C-6 atom out of plane, enter hepatocytes while “skipping” the lysosome stage, thus improving the drug’s therapeutic effect [51].
The effect of the configuration of the chirality center of the carbohydrate fragment has also been investigated; some studies show that α-glycosides have a higher activity towards the model plant receptor ConA [52] or cell line HepG2 derived from hepatocytes [53], in comparison with β-glycosides.

In addition, the use of liposomes as carriers requires a minimal effective share of carbohydrate ligands in the total lipid content; it is called the threshold effect [54]. In vitro data for the binding of D-mannose modified liposomes (4a–g) to the plant lectin ConA demonstrate that the minimal effective share of D-mannose is 28% for short spacers (2 oxyethylene units), whereas it is only 3% for a medium-long spacer (6 units).

In the case of D-lactose based glycolipids with a hydrophobic unit that contains two alkyl chains and a succinic acid residue acting as a small linker, the threshold effect is observed upon addition of 5% of the resulting substance to the liposome composition [55]. In addition, there is evidence of a sharp increase in the uptake of carbohydrate-containing particles by macrophages after interaction with Gal/Fuc-recognizing receptors on their surfaces, if the modification rate reaches 50% [56]. The introduction of structuring lipids, such as cholesterol, into the liposomes decreases the carbohydrate threshold, and the use of unsaturated phosphatidylcholines as a lipid matrix increases it [57].

It is worth noting that oxyethylene groups, which are not found in nature, are able to replace natural monosaccharide residues, imitating a long polysaccharide chain. This effect has been established in studies on binding efficiency for three different glycolipids with the plant lectin RCA1 – binding efficiency increased in a series of aliphatic derivatives of D-galactose, D-lactose, and D-galactose, with a short hydroxyethylene spacer attached to it [43]. In addition, the use of longer polyethylene glycol chains (more than 10 units) creates the effect of steric surface protection of liposomes from blood proteins, leading to prolonged blood circulation of the complexes. An increase in ligand content in the lipid composition results in easier penetration into liver cells, and the presence of a long hydrophilic spacer slows down the removal of the complexes from the blood [44].

Hydroxyl groups at the terminus of a carbohydrate-containing ligand may facilitate the contacts between the modified liposome and the active site of ASGP-R, as well as improve the transfection activity of the complexes of such vesicles with nucleic acids. A number of in vitro studies on cationic liposome bioactivity, where the polar head of the lipid contains a hydroxyl group (5a–d), have shown that complexes formed with DNA are more stable, due to the formation of hydrogen bonds between the surface of the bilayer and the molecules of the nucleic acid [58]. The closer these groups are to the positively charged quaternary nitrogen atom (5a), the more effective complex formation is [59].
An increase in the number of hydroxyl groups in cationic lipids also improves transfection efficiency [60]. Hydroxyl groups at lipid termini within the bilayer perform a function that is analogous to that of PEG (polyethylene glycole) chains, forming a small protective layer around the liposome, thus letting the glycoside-containing particles further circulate in the blood, in comparison with cationic dispersions [50].

In summary, the crucial parameters in the design of such ligands are spacer length, hydrophilic-lipophilic balance of the molecule, and its spatial geometry.

**Success in developing liposomal medications for liver disease therapies**

Cationic liposomes that contain analogs of natural lipids may have much higher efficacy of gene delivery, compared to liposomes based on phospholipids, thanks to their particular bilayer structure [61]. However, such conjugates require the presence of helper lipids that play an important role in lipoplex formation from cationic liposomes and nucleic acids, and determine their morphology [62]. One study suggests aliphatic esters of saccharose as helper lipids, where the hydrophobic domain is represented by residues of various fatty acids (6a–c). They have shown high efficacy by improving transfection activity of lipoplexes in vitro and in vivo [63]. The cellular uptake of modified cationic liposomes increased by 20–30%, whereas cytotoxicity decreased by 20–60%. However, the structure of such esters may have a great impact on the transfection efficiency and liposome toxicity; this is why the choice of length and type of fatty acid residue plays an important role. The existing data shows that liposome size gradually decreases with an increasing hydrophilic-lipophilic balance. On average, liposome diameter is lower for those particles that contain lauric acid residues (6a) than the diameter of liposomes with stearic esters of stearic acid (6b), even with the same hydrophilic-lipophilic balance.

The length of the hydrophobic fragment may affect the stability and fluidity of liposomes as well. It has been shown that alkyl chains with 12 carbon atoms provide the best penetration into cells, in comparison with shorter (6–10 atoms) or longer (>14 atoms) chains [64]. Saccharose esters with short chains (lauric acid residues), whose hydrophilic-lipophilic balance is equal to 6, allow for the formation of liposomes, which provide high transfection efficiency for DNA plasmids and suppress tumor growth in mice [65].

There are a number of studies, which demonstrate that an increase in transfection efficiency and genetic silencing in liver cells may be achieved even by simple conjugation of the glycoside with a DNA or RNA molecule. It has been shown that the targeted delivery of genetic material for HCV treatment, when such a modification is used, increases the penetration of nucleic acid into the cells by 10 times [66]. The conjugation of an antisense oligonucleotide with even one GalNAc residue (7) significantly increases the efficiency of the delivery of the bioactive molecule. In this case, lysine has been used as a spacer and branching agent.

[Diagram of structures 6a–c and 7]
Apart from gene therapy agents, chemotherapy drugs have been quite successfully targeted into liver cells as well. In one study, cationic liposomes carrying doxorubicin were coated by D-galactose residues, at the stage of liposomal carrier formation. In vitro cytotoxicity experiments have shown that delivery to Huh-7 cells (human hepatocarcinoma) is selective; the cells have ASGP-R on the surface. The cytotoxicity is dose-dependent, increasing with a growing concentration of liposomes in the well of the plate [66].

In order to create another targeted anti-tumor drug, liposomes with encapsulated oxaliplatin have been engineered. Their surface was coated with lactobionic acid, a disaccharide polyhydroxy acid. The cytotoxic agent itself and the unmodified, carrying liposomes were used as comparator drugs. Fluorescent labelling allowed to detect that addition of carbohydrate onto the liposomal surface led to three-fold growth in vesicle concentration in hepatocellular carcinoma cells [68]. Oxaliplatin carried by targeting liposomes had a stronger cytotoxic effect on these cancer cells, in comparison to a “simple” drug and unmodified liposomes.

Confocal microscopy with contrast organoid staining allows the accumulation of liposomes in targeted cells to be investigated. The conjugation of D-galactose residues with the surface of cationic liposomes via the amino group of the DSPE lipid (8) leads to a significantly better uptake by HepG2 hepatocytes [69].

The co-incubation of cells with liposomes and specific conjugation inhibitors, such as indomethacin and chlorpromazine, has shown that adding these substances dramatically decreases the number of modified liposomes inside the cells [69]. It is known that chlorpromazine blocks clathrin-dependent endocytosis, and that indomethacin is caveolin-dependent. This is why the study confirms that penetration of carbohydrate-containing particles into hepatocytes occurs via a clathrin-mediated mechanism, and also shows that caveolin-dependent uptake, too, plays an important role. The latter process is responsible for the uptake of the majority of complex microorganisms and viruses.

There has been research on cationic liposomes containing D-galactolipid and the POPC helper lipid (9) in various ratios [70].

In vitro experiments have shown that liposome uptake by Huh-7 cells increases with a growing amount of a carbohydrate-containing lipid in the lipid matrix. Carbohydrate-containing liposomes resulted in nearly two times better silencing of VEGF genes, responsible for the development of squamous epithelium tumors, in comparison to “standard” cationic liposomes. The introduction of a glycolipid into the liposome, if that glycolipid has been obtained by a reaction between lactose and DOPE lipid (10), does not require any helper lipids for a successful exit from endosomes.
An in vivo pharmacokinetics study has shown that lipoplex uptake by liver cells occurs through a receptor-mediated mechanism, since just 5 minutes after the start of the experiment the majority of the modified complexes were found in the liver, whereas “standard” cationic liposomes remained in the plasma for the most part [70].

Another in vivo experiment with BALB/c mice has demonstrated the faster excretion of glycated liposomes, in comparison to cationic liposomes based on phosphatidylcholines and cholesterol [71]. For this study, two cholesterol-based glycoconjugates were synthesized; they contained D-galactose (11a) or N-acetyl-D-glucosamine (11b). In 20 minutes after the start of the experiment, the modified liposomes were almost absent in the blood, but were found in the liver, spleen, and kidneys. Additionally, liposomes with D-galactose residues on the surface reached the liver from the blood faster.

![Chemical structures](image)

11a, b

The synthesis of D-lactose based glycolipids has been reported; the molecules had spacers of various lengths, based on di-, tri-, and polyethylene glycol. These compounds (12a-f) were added to lipids, in the amount of 5%, during bilayer formation, after which the efficiency of binding of all three modified liposomes to the RCA1 receptor was evaluated [72].

It was expected that the longest spacer, PEG (12c, f), would provide the highest affinity of the complexes, due to the longer distance between the vesicle surface and the carbohydrate fragment. However, the in vitro results indicated that the most successful ligands were the ones with a triethylene glycol spacer (12b, e) [72]. The authors speculated that the PEG chains were too long, becoming a barrier between the receptors and the carbohydrate-containing ligands, thus preventing them from interaction. The same experiment was performed with a D-lactoside (13) containing 7 carbohydrate residues linked with a lipopeptide through a 1,2,3-triazole cycle [73]. The results confirmed that polyvalent glycolipids also have good potential for generating targeted, modified liposomes.
Glycolipid 13 was used as a ligand in the structure of a cationic liposome to study its effect on the transfection efficiency for the HepG2 cell line (human hepatocellular carcinoma). Almost the same glycolipid, but with a single D-lactose residue, was used as a different ligand type. It was found that the branched ligand decreases transfection activity of the liposomal complex that carries the plasmid, due to steric hindrance and a shielding effect [74].

Studies of glycolipid-containing liposomes with a triazole cycle in the structure are presented in both Russian and foreign literature, indicating that such a link between the lipophilic and the hydrophilic fragments is convenient [23, 75]. In addition, D-galactose derivative 14 contains a benzene ring as a linker [75].

Conclusions

Liposomal delivery systems are well suited for carrying anti-tumor drugs as well as nucleic acids. They allow us to design stable and effective medications with fewer side effects, compared to “pure” active molecules. However, such delivery systems may cause toxic and immunological effects, due to unselective particle distribution in the body and the relatively large size (compared to other delivery systems), thus
activating a protective response. Apart from this, lipoplexes do not release their contents after entering the cell very effectively. It is necessary to achieve targeted drug delivery to the organ of interest by adding specific ligands to the liposomal surface. Glycolipids based on D-carbohydrates, when in the liposomes, facilitate penetration into liver cells by a receptor-mediated, clathrin-dependent endocytosis mechanism, which is activated upon contact of the carbohydrate-containing ligand fragment with the active site of the asialoglycoprotein receptor (ASGP-R). This can be addressed via the use of monovalent derivatives of carbohydrates as well as polyvalent glycoconjugates. Alterations in the ligand structure and the number of liposomal modifications can boost the therapeutic effect. The distance between the liposomal surface and the carbohydrate residue (spacer length), as well as the hydrophilic-lipophilic balance of the ligand molecule, have a great effect on affinity and the cellular response.

In summary, to ensure the minimal efficiency of the interaction between a modified particle and asialoglycoprotein receptor. It is required to add 5% of a specific ligand to the liposome, and the distance between the liposomal surface and the carbohydrate residue should be approximately 10 Å. The presence of glycolipids in a cationic liposome accelerates penetration into the cell, not only due to the receptor-mediated mechanism, but also because of the physicochemical changes in the particle’s surface. A number of studies have confirmed that modified liposomal complexes exit the endosomes more easily than “standard” cationic liposomes; we can speculate that glycolipids play a helping role as well. Carbohydrate-containing liposomes are able to effectively deliver both genetic material and cytotoxic drugs into target cells, indicating that it is possible to use such compositions against a wide range of liver diseases.

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