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Drug targeting hepatocytes specifically Asialoglycoprotein receptors

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ABSTRACT

The Asialoglycoprotein receptor has been on interest to target the liver for treatment of multiple ailments. Asialoglycoprotein receptors being easy target for carbohydrate molecules, contains a calcium ion channel, type C-lectins and are major on the hepatocyte (the outer covering of liver plasma membrane). This receptor has a great potential, it can help target a drug onto a specific liver site, it can be used for diagnostic and therapeutic purposes.

When we discuss about liver diseases with a very minimal amount of nontargeting drug or a delivery system, is due to the high specificity, effective internalisation rate and also easy access to the blood and lymph vascular system. But then the targeting is not achieved as the drug can be carry forward with the flow of blood. We in this review article draw a conclusive structure of the number of molecules which can impact on targeting liver. Along with its use in liver diagnostics, it also examines the receptor's clinical importance in a variety of liver diseases.

Graphical abstract

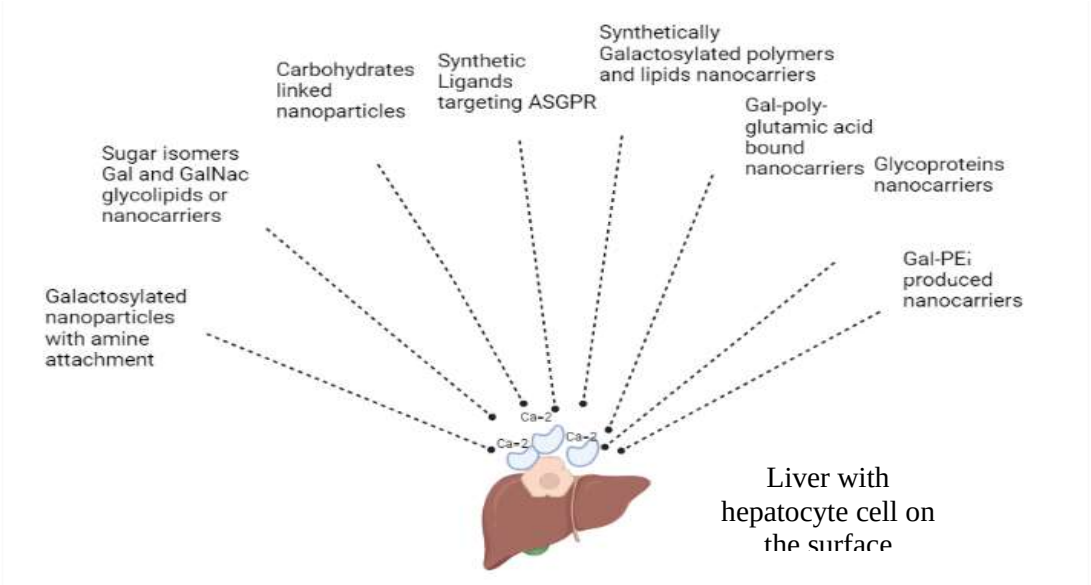


Figure 1: Different procedurs or ligands to target Asialoglycoprotein receptor

1. INTRODUCTION

Liver is a vital organ of our body, the proper functioning, and maintenance of a disease-free state becomes the task. The liver is prone to several disorders as it is the major site of metabolism and any changes or harmful exposure can lead to serious disorders like cancer. Liver, a major site for defence system by reticuloendothelial system (RES) and phagocytosis. Hepatic disorders and diseases, 5th major cause of death and are more evolved in the developing countries, reflect a consistent increase

[1]. In view of drug delivery, it has been eloquent that the drugs which consists of molecular weight more than 300Da show better absorption, it accrues in the liver and the rapid transit by P-glycoprotein and elusive target cell presence decreases its acceptance medically. Hepatic disorders are so lethal that the hepatocellular carcinoma (HCC) is termed as third most lethal malignancy in underdeveloped nations [2]. This also creates the need for druthers to find better treatment or targeting ways to hepatic ailments. The arduous of the need can be understood as nearly one child per minute dies from malaria, which is frequently connected to relapse and nonspecific liver targeting. It's interesting that these conditions only affect one type of liver cells, the hepatocyte [3].

Liver specific delivery or targeting is still a hot topic, it requires the focus of recent reviews. The recent research idea of targeting a cell for endocytosis is exiting as the receptors bind to only a specific ligand and this allows a wide expectation of research towards molecular basis of the organ. The chances of higher amount of drug delivery to the sole organ and cells with the minimal loss of the drug and drug toxicity is reached when receptor targeting is followed. It also increases the efficacy and decrease the side effects and wide distribution. For targeted administration, choosing the right receptor could significantly boost the chances of success [4]. Targeting the abundant hepatocyte prevalent Asialoglycoprotein receptor (ASGPR), exiguous on extrahepatic tissues may offer a desirable benefit for hepatic treatment by hepatocyte-mediated administration. Carbohydrate or carbohydrate linked nanocarriers enabling targeted delivery to specific cell types are indeed the focus [5]. Admitting the fact that targets mentioned, including hepatocytes are highly discussed here, carbohydrate or carbohydrate linked nanocarriers are the main emphasis.

2. ASIALOGLYCOPROTEIN RECEPTOR

The Ashwell-Morell Receptor (AMR), or ASGPR, was the first mammalian receptor to have affinity towards the carbohydrates. It consists of an exposed non-reducing D-galactose (Gal) or N-acetylgalactosamine (GalNAc) as end groups, ASGPR clears desialylated glycoprotein. ASGPR also can absorb serum immunoglobulin-A, hepatic lipoproteins, cellular fibronectin, and prothrombotic substances [6]. Some infections that affect hepatocytes located in the liver are also due to cell components interaction with that of ASGPR.

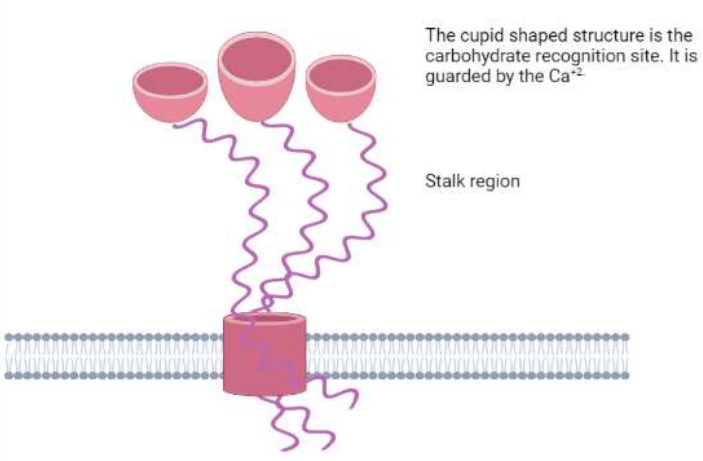


Figure 2: Structure of ASGPR

Functions of ASGPR

ASGPR is grouped structure of glycoprotein which are soluble in water such as albumin or albumin derivatives, along with carbohydrates and sialic acid. Major 48 kDa (ASGPR-1) and small 40 kDa (ASGPR-2) subunits combine to create ASGPR. Asialoglycoproteins, which contain terminal galactose or N-acetylgalactosamine residues, are primarily bound, internalised, and then cleared from circulation by ASGPR [7]. Ca2+, the location of terminal galactose residues, and a pH optimal above 6.5 are all necessary for the binding of ligands to ASGPR. ASGPR-deficient mice exhibit reduced asialoglycoprotein clearance but do not accumulate glycoproteins in the serum, indicating that ASGPR is not the main factor controlling blood levels of glycoproteins [8].

The elimination of apoptotic cells, the removal of low-density lipoprotein (LDL) and chylomicron remnants, the disposal of cellular fibronectin, and the clearing of IgA from circulation have all been linked to ASGPR [9]. Recent evidence is consistent with the idea that hepatotropic viruses employ ASGPR to enter hepatocytes. Additionally, there is proof that ASGPR takes role in the removal of activated lymphocytes [10].

Assertation of ASGPR

Hepatocytes exhibits ASGPR on the site which is communicated between nearby cells or faces the sinusoids, that are

fenestrated blood vessels in use for material exchange between blood and cells. Additionally, it is also present at the outer surface of hepatic cells, peritoneal macrophage sac, rat and human testicles, human sperm, human intestinal epithelial cells, and peripheral blood monocytes. ASGPR has also been shown to be expressed in colorectal cancer that metastasized from the liver. However, ASGPR expression on hepatocytes is significantly higher than that in other parts of the body [11]. High expression of ASGPR on the hepatocyte site, it has no or less presence at other sites, and it should easily get into the blood vessel and also be readily distribute these are attractive characteristics which allows faster targeting and less amount of toxicity for ASGPR target. Hepatic ASGPR expression is destructed by some of the hazardous substance such as alcohol, carbon tetrachloride, lipopolysaccharide, and anti-Fas antibody [12]. ASGPR dysfunction is also linked to diabetes and partial hepatectomy. Different cell lines express ASGPR in vitro.

Ligand binding site to ASGPR

ASGPR is made up of two different poly-amino acid chain subunits, measuring 46 and 50 kDa. The internal signal membrane contains a amino terminus in the cytoplasm by a interceded neck part in each of the C-type II transmembrane glycoprotein that make up the component [13].

As discussed, ASGPR is a calcium dependent channel which has the affinity to the carbohydrate, it is also called as CRD (Carbohydrate recognition domain), surround the carboxylic group (COOH) terminal [14]. For ASGPR and glycopolymers to interact, Ca^{+2} is necessary. In ASGPR, of the three Ca^{+2} binding sites one of it on the CRD has a poor binding affinity. Co-ordinate bonds between the oxygen atom in the ligand and the receptor Ca^{+2} have an interatomic distance of 2.8 to 3.0 [15].

ASGPR, has the highest affinity to the Gal and GalNAc, so, sometimes it is referred to as the galactose receptor or the hepatic proteins binding carbohydrates. The H1 subunit of ASGPR's centroid includes the amino acids aspartic acid 241, aspartic acid 265, asparagine 264, glutamic acid 252, glutamine 238, and tryptophan 243, which creates the active site for ligand binding. Co-ordinate bonds promote the interaction between the oxygen atom of the ligand and the calcium ions present on the receptor [16]. The amide and carboxylate side chains of the receptor force the 3- and 4-hydroxyl groups of Gal into hydrogen bonds. Additionally, when Gal binds to ASGPR, it interacts hydrophobically with the tryptophan243 C3, C4, C5, and C6 atoms of ASGPR and forms four hydrogen bonds with the H1 subunit [17]. Due to their sugar dimers, natural ligands like arabinogalactan and pullulan have more than 10 hydrogen bonds. An insilico three-dimensional configuration of ASGPR subunits has also been predicted after binding to a tri-antennary oligosaccharide [18].

Embody of ASGPR

ASGPR interacts with ligands in plasma membrane clathrin-coated vesicles and internalises ligands by clathrin-enabled receptor-mediated endocytosis. It crosses the lipid bilayer of the hepatocyte. Triskelion clathrin-1, a cytosolic coat protein, polymerizes with the assistance of adaptor/assembly proteins, namely assembly protein complexes 1, 2, and 180, to generate clathrin-coated pits, which facilitate internalisation by forming a polygonal lattice on the cytosolic surface. When there is Ca+2 present and the pH is more than 6, Gal/GalNAc binds to ASGPR [16]. ASGPR ligand recognition and binding results in the location of clathrin-coated pits near the budding membrane. After binding, ASGPR internalises quickly, with a half-life of around 3 minutes and a first-order rate constant of 3.4 x 10-8M [16]. When cells are moving from the plasma membrane or trans-Golgi network, the assembly protein helps connect the clathrin scaffolds and receptors.

Ways to target the ASGPR

Asialoglycoproteins are glycoproteins that have lost their sialic acid residues, a type of sugar modification found on the surface of many cells. The removal of sialic acid from the glycoprotein can change its properties, such as its ability to interact with other molecules.

Several methods can be used to target asialoglycoproteins, including the use of lectins, which are proteins that bind specifically to certain sugar residues. For example, the lectin Galectin-3 binds specifically to asialoglycoproteins and has been shown to play a role in cancer progression [19]. Some researchers are also exploring the use of drugs that inhibit enzymes involved in the removal of sialic acid from glycoproteins, as a way to target asialoglycoproteins [20]. It's worth noting that not all asialoglycoproteins are disease-related, they also have important physiological roles in the body, such as in the immune system.

The molecules containing or bound by the sugar ligands can easily bind to the agpr. ASGPR binding is much galore in sugars with a non-reducing terminal such as Gal or GalNAc. Especially, when we look at the affinity with the GalNAc it is 10-50 folds higher compared to the Gal. In the case of directed distribution to hepatic cells, sugars can be quite important. Human serum albumin (HSA) lactosylated (Lac) was used to transport naproxen, and mannose was substituted for Lac to promote high kupffer cells (KC) uptake [21].

Ligands Binding ASGPR

Organic or synthetic sugars and glycoproteins are ligands for ASGP-high-affinity Rs. It is widely known that the galactosides Gal, GalNAc, and others bind exclusively to the ASGP-R. Tri- and tetra antenna containing oligosaccharides possess greater

fondness to ASGP-R than two antenna containing oligosaccharides, according to in silico studies. At a mutual distance of 20, tri antenna containing GalNAc possessed highest fondness towards ASGP-R [22]. Therefore, even little modifications to ligand form result in a significant reduction in binding affinity. To understand the binding affinity of GalNAc, the study of catechism towards physicochemical parameters such as isomer form, branches, division and density of galactose, geometrical arrangement within the ligand, calcium affinity of the ligand and the HLB ratio or balance initially helps to understand the structure [23]. Some ligands are very responsive and are triggered to inflammatory responses which serves as antibiotics.

Glycoproteins and carbohydrates ligands

In nature, there are numerous membrane-bound and soluble glycoproteins and glycolipids, many of which feature terminal Gal and GalNac residues. Asialoorosomucoid (ASOR) and asialofetuin (AF), the study has been carried out on both the structures to identify its affinity towards the ASGPR and protein gene delivery [24]. In contrast to galactose, the cationic glycoprotein lactoferrin which can bind to iron is more profound to the ASGPR binding and so targeting hepatocytes.

The ASGPR targeting carbohydrates containing structures such as lactose, galactosamine, dextran, lactobionic acid (LA), and sterylglucoside (β-Sitosterol—D-glucoside), have been proved to show the affinity towards liver. ASGPR ligands, Polysaccharide structures which are recently being perceived has the easy acceptance and degradation in the body. Hyaluronic acid, pectin, arabinogalactan (AG), and pullulan are ASGPR targeting polysaccharides. AG, is a galactose-based polysaccharide with a high Gal density. The AG structure with 80% mol density, was internalised by hepatocytes 14 times more quickly than AF [25]. The oral administration of AG is generally safe but the repeated administration of IV aggravates toxicity concerns. This is due to the decreased dissociation rate, and also the accumulation of dissociates and degradation in hepatocytes [26]. Now looking towards the Pullulan, which is a glucose-based polysaccharide, show inability to deliver the drug into hepatocyte as it shows lower targeting, it also has dose dependency which makes it less effectual when compared to AG. The other disadvantage is the impotency of it to discriminate between the Gal and D-glucose. The final outcome of the research suggests that the effect of AG and pullulan is reduced in presence of AF.

S. No	Ligands Binding ASGPR	Subunits
1.	Sugar Isomers	Galactose [27]
		N-acetyl galactose [28]
		Glycolipids containing open and closed cyclic galactose [29]
		Cyclic glycolipids with six methylene spacer units [30]
		Lipids with open sugar heads [31]
2.	Galactose density and branches	Tri antennary [32]
		Oligosaccharides [33]
		Tetra antennary [34]
3.	Glycoproteins	α1-acid glycoprotein [35]
		fetuin glycoprotein [36]
4.	Carbohydrates	Arabinogalactan [37]
		Galactose-based polymer [38]
		Pullulan [39]
5.	Synthetic ASGPR	Gal-poly-glutamic acid [40]
		Gal-HPMAa-b-N-3-guanidinopropyl Meth acryl amide block copolymer [41]
		Laca N-succinyl-chitosan [42]

		Gal-PEI [43]
		GalNAc linked biotin [44]
		Modified lipids [45]

Table 1: Different types of ligands and subunits with approach to target ASGPR

Drug-Ligand conjugate and complexes

These systems are made by directly conjugating or complexing a chemotherapeutic drug with a high-affinity homing ligand. They have been widely used for the delivery of anticancer substances and nucleic acids in cancer therapy. L-lysine polymer (PLL) Despite being the earliest polycation known to form DNA complexes, Poly(L-lysine) (PLL) is promptly excreted from the circulation after intravenous administration. Many natural and synthetic galactose ligands have been added with these polymers to improve the biocompatibility and targeting abilities of these polyelectrolyte complexes. The lectin-directed enzyme-activated prodrug approach, which delivers the active form of the drug to a specific cell type, has shown therapeutic effectiveness in a hepatocellular carcinoma (HepG2) disease model. Gal-NAc-siRNA conjugates and doxorubicin-lactosaminated human albumin (L-HSA) have both been successfully investigated for liver targeting and are now in various stages of clinical research [46]. Conjugates can have certain disadvantages, though, such as the possibility of drug inactivation during chemical conjugation, in vivo instability, nontarget drug release, and rapid clearance. To get around these limitations, redox-responsive and pH-sensitive glycopolymer-drug conjugate NPs that can target and programme drug release in the reductive, acidic tumour microenvironment have been created [47].

Drug Nanoparticles

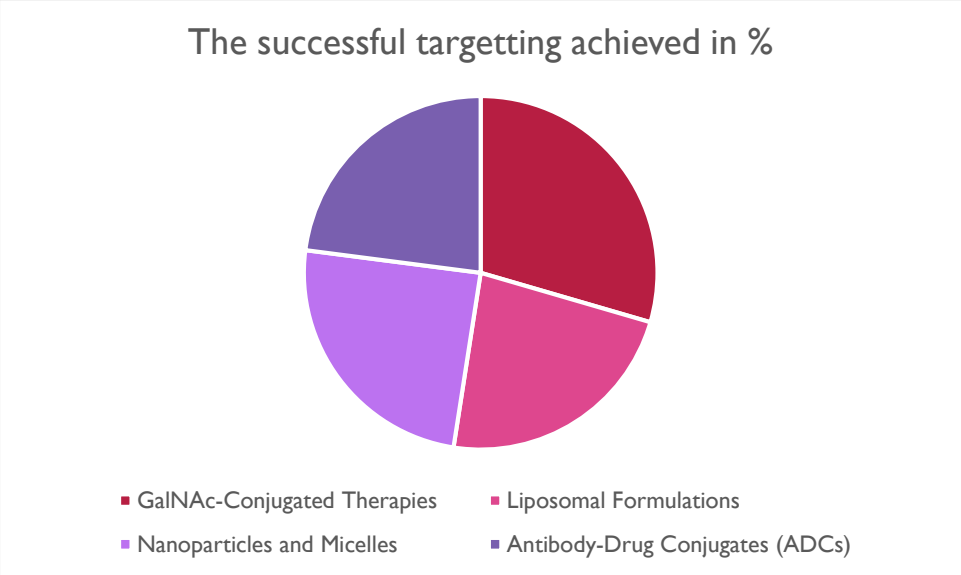
Particle size, surface charge, stealth-enhancing hydrophilicity, and ligand concentration, among other colloidal nanocarrier features, determine absorption, targeting potential, and in vivo destiny. It has been demonstrated that clathrin- or caveolae-mediated endocytosis may take up galactosylated NPs. Notwithstanding differences, it is generally acknowledged that ASGP-R is unable to identify or digest nanocarriers larger than 70 nm. Hepatocytes actively ingested PEGylated-Gal-NPs for gene delivery that were 50 nm in size, whereas KC actively ingested 140-nm particles. As shown by lactosaminated cationic PLL, which achieved 98% hepatocyte uptake following intravenous injection, positively charged nanocarriers have been reported to engage with the negatively charged ASGP-R-binding sites rather easily. Nanocarriers with stealth coatings had better serum circulation and increased hepatocyte absorption [48]. Gal-liposomes and lipoplexes that have been pegylated have shown to have greater hepatocyte uptake than carriers that have not been pegylated; this absorption can be further enhanced by pegylation at higher concentrations. Nanocarriers coated with ASGP-R-specific ligands may slow fast systemic clearance and enhance hepatocyte targeting [49]. Nevertheless, for desirable hepatic uptake, an ideal ligand concentration is required. This is because larger ligand concentrations can produce ASGP-R saturation, which increases the absorption of nanocarriers by other Gal receptors like KC [50]. Higher dosages of GalNAc containing doxorubicin-HPMA conjugate (PK2) resulted in partial ASGP-R saturation. As a stealth agent and an ASGP-R ligand, pullulan and AG are among the few naturally occurring carbohydrates [51].

Benefits and hazards of targeting ASGPR

The ASGP-R-targeted drug delivery systems have failed in clinical trials despite the promising potential they have demonstrated in multiple preclinical research. Clinical failure may have occurred as a result of the receptor's variable performance and expression in different animals and illness stages. Moreover, because the majority of preclinical investigations are carried out on healthy animals, effective targeting might not be a reliable predictor of effective therapy. Due to ASGP-R expression in both healthy hepatocytes and HCC cells, off-target effects have been seen at certain levels. By creating matrix metalloproteinase-2 cleavable DOPE/PEG liposomes for HCC-specific administration. Effective targeting is also significantly hampered by the variable levels of ASGP-R expression that vary with time, disease severity, and stage of development. Moreover, surface-modified ASGP-R delivery systems need difficult preparation procedures, can cause autoantibody formation and immunogenicity, and have serious repercussions.

Different formulations	GalNAc-Conjugated Therapies	Liposomal Formulations	Nanoparticles and Micelles	Antibody-Drug Conjugates (ADCs)	Reference
Number of formulations studied	20	15	30	10	[52]
The successful targetting achieved in %	90	70	60-80	70	[53]

Table 2: showing different formulations used for targeting asialoglycoprotein receptors, the number of formulations which are already being prepared with the technologies and successful targeting percentage.



Graph 1: Pie chart graph representing the extent of successful targeting achieved by different formulations on asialoglycoprotein receptor.

3. CONCLUSION

By developing DOPE/PEG liposomes that are cleavable by matrix metalloproteinase-2 for the treatment of HCC. The fluctuating levels of ASGP-R expression that fluctuate with time, illness severity, and stage of development considerably hinder effective targeting. Surface-modified ASGP-R delivery systems are also time-consuming to prepare, can result in the development of autoantibodies and immunogenicity, and have detrimental effects.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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