Journal of Controlled Release (Review Article)

Glycosylation-mediated targeting of carriers

Shigeru Kawakami[a] and Mitsuru Hashida[b,c,*]

[a]Department of Pharmaceutical Informatics, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan
[b]Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan
[c]Institute for Integrated Cell-Material Sciences, Kyoto University, Yoshida-Ushinomiya-cho, Sakyo-ku, Kyoto 606-8302, Japan

*Corresponding author. Tel. +81-75-753-4525; Fax: +81-75-753-4575.
E-mail address: hashidam@pharm.kyoto-u.ac.jp

Abstract
For safe and effective therapy, drugs should be delivered selectively to their target tissues or cells at an optimal rate. Drug delivery system technology maximizes the therapeutic efficacy and minimizes unfavorable drug actions by controlling their distribution profiles. Ligand-receptor binding is a typical example of specific recognition mechanisms in the body; therefore, ligand-modified drug carriers have been developed for active targeting based on receptor-mediated endocytosis. Among the various ligands reported thus far, sugar recognition is a promising approach for active targeting because of their high affinity and expression. Glycosylation has been applied for both macromolecular and liposomal carriers for cell-selective drug targeting. Recently, the combination of ultrasound exposure and glycosylated bubble liposomes has been developed. In this review, recent advances of glycosylation-mediated targeted drug delivery systems are discussed.

Keywords: macromolecular carriers, liposomes, targeting, glycosylation, drug delivery

Contents

1. Introduction

After the administration of drugs to an individual, they are distributed according to their physicochemical and/or biological properties. Subsequently, they are eliminated from the body. For safe and effective drug therapy, they should be selectively delivered to their target tissues or cells at an optimal rate. Drug delivery systems are a technology that maximizes drug therapeutic efficacy and minimizes their unfavorable actions by controlling distribution profiles. Thus, controlled drug disposition is an important factor that determines the therapeutic efficacy of drugs. Drug delivery systems have been used clinically, particularly for anticancer drugs and are essential for the practical application of recombinant protein and nucleic acid medicine.

One major approach of targeting is the use of drug carriers that possess an affinity towards a population of target cells. To date, many macromolecules and lipid dispersion preparations, i.e., liposomes and emulsions, as well as various natural and synthetic macromolecules have been developed as drug carriers. Important characteristics of drug carriers include: i) biocompatibility, ii) lack of toxicity and immunogenicity, iii) biodegradability or minimal accumulation in tissues or organs, iv) adequate functional group for chemical modification of ligands and/or drugs, and v) productivity [1,2].

Ligand-receptor binding is a typical example of a specific recognition mechanism. Therefore, ligand-modified drug carriers have been developed for active cell-selective drug delivery by receptor-mediated endocytosis. So far, galactose, mannose, fucose, mannose-6-phosphate, sialyl Lewis x, peptides, and proteins (i.e., transferrin, epidermal growth factor, and anti-human epidermal growth factor receptor 2 antibodies) have been used as ligands [3-10]. Among the various ligands reported, sugar recognition mechanisms are a promising approach for active targeting because of their high affinity and expression.

Recently, various nucleic acids have been developed for medication including pDNA, antisense DNA, small interfering RNA (siRNA), and microRNA [11-15]. The receptor-mediated targeting by glycosylation has been used for therapy with nucleic acids. In the case of nucleic acid medicine, not only recognition by receptors but also intracellular fate, i.e., endosomal escape, should be considered. To solve this problem, multifunctional glycosylated particulates and a combination method of glycosylated bubble particles with ultrasound exposure have been developed. This review focuses on the recent progress of glycosylation-mediated targeting of low-molecular-weight drugs and nucleic acid drugs by drug carriers using macromolecules and liposomes.

2. Distribution characteristics of macromolecules

The distribution of macromolecular carriers is determined by their physicochemical properties, such as electric charge, molecular size, and hydrophilic/hydrophobic balance. Although ligand modifications with a high affinity to target cells are essential for macromolecular carriers in active targeting, the physicochemical properties of macromolecular carriers have an important function in determining the targeting efficacy of ligand-modified macromolecular carriers. The optimal design of macromolecules that possess suitable physicochemical properties of distribution following their systemic administration should be considered for precise active targeting by glycosylated macromolecular carriers.

2.1. Overcoming the rapid clearance of macromolecular carriers

Prolonged retention of drug-ligand-modified macromolecular carrier conjugates in the blood promotes their distribution to target cells. The controlled physicochemical properties of macromolecular carriers are an important factor for their prolonged retention and are based on the biological characteristics present in tissues and organs. These distribution characteristics and pharmacokinetic analysis of macromolecules have been summarized in our previous review [1, 2, 18, 19].

The kidney has an important role in the elimination of drugs from the body. Therefore, the reduction of glomerular filtration in the kidney should be considered. Macromolecules with a molecular weight of < 50,000 (approximately 6 nm in diameter) are suitable for glomerular filtration. In addition, the surface charge of macromolecules affects the reabsorption process in the kidney. For example, positively charged dextran (70 kDa) has higher glomerular permeation via endocytosis than negatively charged dextran although they have a similar molecular weight [20].

The liver also has an important role in the fate of drugs in the body, and thus the reduction of liver uptake should be considered. Our group previously showed that positively charged macromolecules (bovine serum albumin (BSA) and dextran) were distributed to the liver according to their contact area in the parenchymal and non-parenchymal cells via electrostatic interactions [21, 22]. In contrast, negatively charged BSA and dextran were distributed to the liver non-parenchymal cells via scavenger receptor-mediated endocytosis [23-25].

It is necessary to consider the general fate of macromolecules from the viewpoint of their physicochemical and biological characteristics when rationally designing ligand-modified macromolecular carriers for active targeting.

3. Glycosylated macromolecules for cell-selective targeting

Table 1 An example of glycosylation-mediated targeting of macromolecular carriers

Target cells	Ligand	Macromolecular carriers	Delivered compounds	Ref.
Liver PC	Galactose	Poly(L-lysine)	ara-AMP	29
Liver PC	Galactose	Poly(L-lysine)	pDNA	30
Liver PC	Galactose	Poly(L-glutamic acid)	prostaglandin E_1	35
Liver PC	Galactos e	Dextran	araC	28
Liver PC	Galactose	PEI	pDNA	38
Liver PC	Galactose	Chitosan	pDNA	43
Liver PC	Galactose	Dendrimer/α-cyclodextrin	pDNA	48
Liver NPC	Mannose	Poly(L-lysine)	Antisense DNA	63
Liver NPC	Mannose	Poly(L-lysine)	pDNA	64
Peritoneal macrophages	Mannose	Chitosan	pDNA	71
Dendritic cells	Mannose	Chitosan	pDNA	72
Dendritic cells	Mannose	(Biodegradable) PEI	pDNA	70
HSC	M6P	HSA	doxorubicin	86
HSC	M6P	HSA	gliotoxin	87
HSC	M6P	HSA	losartan	89
HSC	M6P	HSA	Y27632	90-92
HSC	M6P	BSA	TFO	95,96
Cancer cells	M6P	HSA	doxorubicin	94

PC: parenchymal cells
NPC: non-parenchymal cells
HSC: hepatic satellite cells

Table 1 summarizes an example of cell-selective targeting with macromolecular carrier systems based on glycosylation. Glycosylation-mediated targeting is a promising approach for cell-selective targeting because it has specificity for target cells and productivity for standardization. In this case, the controlled distribution of non-modified macromolecular carriers determined by physicochemical properties is important for cell-selective targeting. In addition, the density of ligands in macromolecular carriers might affect recognition by the receptor. Fig. 1 summarizes design of glycosylated macromolecular carriers.

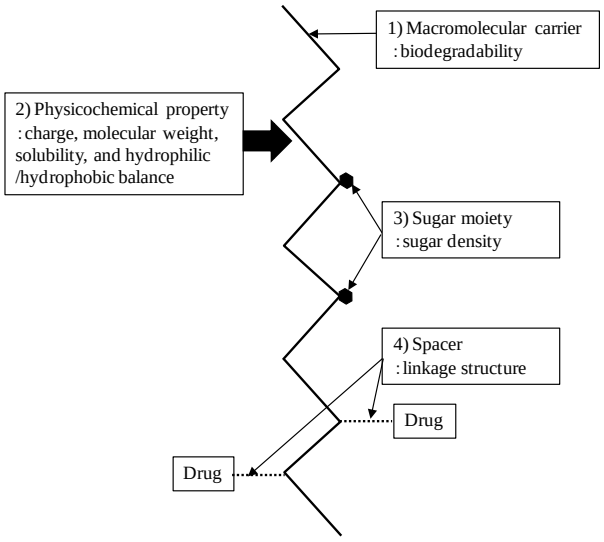

Fig. 1 Design of glycosylated macromolecular carriers

3.1. Galactose modification

Asialoglycoprotein receptors are exclusively expressed on hepatocytes and have a high affinity and rapid internalization rate. Therefore, galactosylated macromolecules

have been studied for hepatocyte-selective drug targeting. Discontinuous capillaries (sinusoidal capillaries) are commonly observed in the liver [26], and contain large interendothelial junctions with a mean diameter of 150 nm [26, 27]. Intravenously injected galactosylated macromolecules must pass through the sinusoidal capillaries to reach the hepatocytes expressing asialoglycoprotein receptors for recognition. To date, dextran [28], poly-L-lysine (PLL) [29-33], poly-glutamic acid (PLGA) [34-36], polyethlenimine (PEI) [37-42], chitosan[43-47], dendrimer/α-cyclodextrin conjugate [48], and their derivatives [49, 50] have been studied for galactosylated macromolecular carriers for hepatocyte-selective targeting.

The design of carrier types based on the physicochemical properties and galactose density of the carriers are of significance for targeting efficacy by galactosylated macromolecular carriers. Our group previously compared the targeting efficiency between positively charged galactosylated PLL and negatively charged galactosylated PLGA in mice [51]. After intravenous administration, galactosylated PLGA was selectively taken up by liver parenchymal cells and was significantly inhibited by the co-administration of excess galactosylated BSA, suggesting the asialoglycoprotein receptors mediated uptake of PLGA. Galactosylated PLL showed a higher accumulation in the liver compared with unmodified PLL. However, the effect of galactosylation on liver parenchymal cells-selective targeting was less pronounced than for galactosylated PLGA. These results suggest that the selection of macromolecular carriers is important to achieve efficient cell-selective targeting.

The galactose density of macromolecular carriers might affect targeting efficacy. Our group studied the effect of galactose moiety surface density on galactosylated proteins such as superoxide dismutase (SOD) and BSA. After intravenous administration in mice, galactosylated proteins were recovered in the liver and were shown to be highly dependent upon the degree of galactose modification. Thus, targeting efficacy can be controlled by the degree of macromolecular carrier galactosylation.

Hepatocytes are notable targets for gene and nucleic acid therapy, because many refractory metabolic diseases are caused by hepatocyte-derived proteins. In addition, hepatocytes are considered targets for the production of therapeutic proteins to secrete into the blood because the liver possesses a rich blood flow. The asialoglycoprotein receptor-mediated targeting of pDNA and/or nucleic acids to hepatocytes using galactosylated PLL, galactosylated chitosan, galactosylated PEI, or galactosylated dendrimer/α-cyclodextrin conjugate was previously investigated. To achieve high gene expression by galactosylated polycations/pDNA complexes, a rationally designed delivery system is needed for optimal pharmacokinetics including: i) condensation of

pDNA for the penetration of fenestrae, ii) pharmacokinetic processes of pDNA to hepatocytes by asialoglycoprotein receptor recognition, and iii) release of pDNA from endosomes/lysosomes into cytoplasm after internalization. Of these, the release of pDNA from endosomes/lysosomes into the cytoplasm after internalization is a major obstacle for efficient gene expression in hepatocytes. Therefore, manipulation of this process by functional peptides or the use of PEI with pH buffering functions in lysosomes [53] by galactosylated macromolecules might allow high transfection rates *in vivo* [40, 49, 54-56].

3.2. Mannose modification

Macrophages have an important role as effector cells in inflammation and antigen presentation. Mannose receptors are expressed on Kupffer cells, alveolar, peritoneal, and splenic macrophages, dendritic cells, and a subset of vascular endothelial cells [57-61]. Therefore, mannosylated macromolecules have been studied for mannose receptor-expressing cell-selective targeting. Similar to galactosylated macromolecular carriers described above, dextran [28], PLL [62-64], PLGA [65], PEI [66-70], chitosan [71-73], and dendrimer/α-cyclodextrin conjugate [74, 75] have been studied for mannosylated macromolecular carriers to selectively target mannose receptor expressing cells. The design of carrier types based on the physicochemical properties and mannose density of the carriers is of significance for targeting efficacy by mannosylated macromolecular carriers.

Our group developed a mannosylated macromolecular carrier system for cell-selective targeting based on dextran, which has high solubility, an abundance of hydroxyl groups for chemical modification, low immunogenicity, and is used in the clinic as a plasma expander [2]. Our group studied carboxymethyl dextran to minimize the interaction with endogenous components and then coupled them with mannose moieties as a ligand [28]. After intravenous injection into mice, mannosylated carboxymethyl dextran was rapidly eliminated from the plasma and accumulated in liver non-parenchymal cells including Kupffer cells and liver endothelial cells that expressed mannose receptors. When we compared the targeting efficiency between positively charged mannosylated PLL and negatively charged mannosylated PLGA in mice [51], the liver non-parenchymal cell selectivity of mannosylated PLL was lower than for mannosylated PLGA. These observations correspond with results observed for galactosylated macromolecular carriers. Thus, the selection of macromolecular carriers based on their physicochemical properties is important to achieve efficient cell-selective targeting.

Similar to galactosylated macromolecular carriers, the mannose density of macromolecular carriers can affect the targeting efficacy. We previously reported the effect of the surface density of mannose moieties in mannosylated proteins such as SOD, BSA, and IgG [76]. After intravenous administration in mice, mannosylated proteins were recovered in the liver and were highly dependent on the degree of mannose modification. These results suggest that targeting efficacy can be controlled by the degree of mannosylation of macromolecular carriers.

Mannose-expressing cells are notable targets for gene and nucleic acid therapy for refractory diseases such as chronic inflammation, cancer, and virus infection, amongst others. Mannose receptor-mediated targeting of pDNA and/or nucleic acids to macrophages was previously reported using mannosylated PLL, and mannosylated chitosan. When considering mannosylation, the condensation of pDNA is not critical because it is not required to pass through fenestrae to reach the target cells unlike galactosylated macromolecular carriers. However, the release of pDNA from endosomes/lysosomes into the cytoplasm after internalization is a major obstacle for efficient gene expression in mannose receptor-expressing cells. To solve this problem, mannosylated PEI, or mannosylated dendrimer/α-cyclodextrin conjugate that can accelerated the cytoplasmic release of pDNA have been investigated.

3.3. Mannose-6-phosphate (M6P) modification

Two M6P receptors, cation-dependent M6P receptors and cation-independent M6P/insulin-like growth factor (M6P/IGF-II), belong to the family of p-type lectins and are involved in the transport of cellular proteins from the cellular surface or trans-golgi network to lysosomes [77-79]. M6P/IGF-II receptors are highly expressed on activated hepatic stellate cells (HSCs) [80-82]. Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen. Excessive collagen is considered responsible for activated HSCs, which constitute 5–8% of liver cells [83].

M6P modification of macromolecular carriers has been studied for cell-selective targeting to HSCs or cancer cells that highly express M6P/IGF-II receptors. M6P modified human serum albumin (HSA) was accumulated in the liver by an M6P-modification-dependent mechanism [84]. After intravenous administration, $M6P_{28}$-HSA (M6P:HSA=28:1) preferentially accumulated in HSCs of fibrotic rats and liver endothelial cells in normal rats. In contrast, $M6P_{28}$-HSA predominantly accumulated in liver endothelial cells in normal rats, suggesting a degree of the fibrosis is required for the M6P modification targeting system. These results demonstrate the importance of targeting efficacy to HSCs determined by the degree of M6P expression

of macromolecular carriers in fibrotic rats. This observation corresponds with galactosylated and mannosylated macromolecular carriers as previously described. In addition, the uptake of M6P-HSA in slices of human liver was reported [85].

HSC-selective targeting by macromolecular carriers has been studied using various drugs conjugated to M6P-HSA. Greupink et al. developed a doxorubicin (DOX)-conjugated M6P-HSA (M6P-HSA-DOX) for HSC-selective targeting of bile duct-ligated rats [86]. They showed that M6P-HSA-DOX was distributed in the liver and immunohistochemical double staining revealed accumulation in HSCs. In addition, M6P-HSA-DOX reduced HSC proliferation by 82% *in vitro*. These results indicate that inhibition of activated HSC proliferation by cytostatic drugs can prevent the fibrotic process. Similarly, Hagens et al. developed glitoxin (GTX), an apoptosis-inducing agent, conjugated with M6P-HSA (M6P-HSA-GTX), which attenuated liver fibrosis in bile duct-ligated rats [87]. Although GTX induced adverse effects on hepatocytes, M6P-HSA-GTX did not show such effects. To date, various drugs including tyrosine kinase inhibitor, angiotensin type 1 receptor blockers, Rho kinase inhibitor, and conjugated M6P-HSA have been developed to inhibit liver fibrosis [88-93]. Prakashi et al. reported tumor-selective targeting by M6P-HSA-DOX through M6P/IGF-II receptors in tumor-bearing mice [94].

Regarding oligonucleotide delivery, Ye et al. developed M6P-BSA conjugated with oligonucleotides via a disulfide bond [95]. A triplex-forming oligonucleotide (TFO) was used to inhibit transcription via interactions with genomic DNA. After intravenous injection, $M6P_{20}$-BSA-[^{33}P]TFO was rapidly eliminated from the blood and a significant increase in uptake by the liver was observed. Study of intrahepatic distribution revealed that $M6P_{20}$-BSA-[^{33}P]TFO was preferentially taken up by HSCs compared with hepatocytes, Kupffer cells and/or endothelial cells. In addition, pre-injection of excess $M6P_{20}$-BSA significantly decreased the liver uptake in normal and fibrotic rats, suggesting M6P/IGF-II receptors mediated the uptake of $M6P_{20}$-BSA-TFO [96]. Zhu et al. developed siRNA-PEG-M6P conjugates for hepatic stellate cell-selective targeting [97]. Although there have been few reports regarding nucleic acid delivery using M6P modified macromolecules, the release from endosomes/lysosomes into the cytoplasm after internalization is a common obstacle for efficient gene expression in HSCs.

4. Distribution characteristics of liposomes

Liposomes have been used in the clinic as drug delivery carriers [98-100]. Therefore, targeted drug delivery by surface modification of liposome ligands is a promising approach for their wide application and delivery of drugs. In general, the

distribution of liposomes is greatly determined by their surface charge, particle size, lipid composition, and dose used. A major factor that determines their rapid elimination is their uptake by the reticuloendothelial (mononuclear phagocyte) system, predominantly the liver and spleen. Phagocytosis of liposomes is accelerated by association with complement and immunoglobulins in the blood. The optimal design of liposomal dosage is one that possesses suitable physicochemical properties, especially avoiding uptake by the reticuloendothelial system upon systemic administration and this should be considered for precise active targeting using glycosylated liposomes.

4.1. Overcoming the rapid clearance of liposomes

After intravenous administration, liposomes are taken up by the reticuloendothelial system in the liver and spleen [101]. Therefore, the reduction and/or blockage of the reticuloendothelial system uptake are promising approaches to enhance the blood concentration of liposomes.

The lipid composition of liposomes might affect the clearance of liposomes depending on the contents and type of lipids present. Regarding the effect of cholesterol incorporation in liposomes, cholesterol-free liposomes and cholesterol-poor (20 mol%) liposomes were cleared more readily from blood circulation than cholesterol-rich (46.6 mol%) liposomes [102]. The predominant factor of clearance from the blood circulation is uptake by the reticuloendothelial tissues. Semple et al. analyzed the *in vivo* association of blood proteins with liposomes composed of saturated phosphatidylcholines and cholesterol [103]. When liposomes composed of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearylphosphatidylcholine (DSPC), and diarachidoylphosphatidylcholine (DAPC) were administered intravenously, liposomes containing DMPC and DOPC, that are liquid crystalline prior to injection, showed relatively long circulation times. In contrast, liposomes composed of DPPC, DSPC, and DAPC that are in a gel state at 39^{O}C, were rapidly cleared from blood circulation and were associated with large quantities of associated blood proteins. The incorporation of cholesterol into DSPC liposomes resulted in their deceased protein binding and enhanced circulation time in blood.

The surface charge of liposomes might also affect the clearance of liposomes. Juliano et al. reported that neutral liposomes containing phosphatidyl choline and cholesterol at a 1:1 ratio and positively charged liposomes containing phosphatidyl choline, stearyl amine, and cholesterol at an 18:2:10 ratio were cleared less rapidly than negatively charged liposomes containing phosphatidyl choline, phosphatidyl serine, and cholesterol at a 1:1:1 ratio [104]. Allen et al. reported that the incorporation of greater

than 2 mol% phosphatidyl serine into liposomes caused their rapid elimination from blood circulation. Daeman et al. reported a different intrahepatic distribution of phosphatidyl glycerol and phosphatidyl serine liposomes [105]. Phosphatidyl serine-containing liposomes were found mainly in the liver (75%), while phosphatidyl glycerol-containing liposomes were found in the liver (40%) and spleen (40%) equally. Regarding intrahepatic distribution, phosphatidyl serine-containing liposomes were distributed between Kupffer cells and hepatocytes equally, while phosphatidyl glycerol-containing liposomes were only taken up by Kupffer cells. Gabizon and Papahadjopoulos reported that increasing the molar ratio of negatively charged phosphatidyl inositol from 9% to 23% did not change the distribution, but an increase from 23% to 41% accelerated the clearance of liposomes [106]. It was reported that cationic liposomes/plasmid DNA (lipoplex) can be selectively transfected to lungs following intravenous administration [107-109]. We previously showed that cationic liposomes containing 1,2-di-O-octadecenyl-3-trimethylammonium propane or N-[1-(2,3-dioleoyloxy)propyl]-N, N, N-trimethylammonium methyl sulfate and cholesterol at a 1:1 ratio that were used for gene transfection were mainly distributed in the lung [110]. This is consistent with the transfection characteristics of lipoplex. Therefore, the selection of lipid composition is important when designing targeted drug delivery systems using glycosylated liposomes.

Surface modification of liposomes by ganglioside GM_1 [111] and poly(ethylene glycol) (PEG) [112, 113] could achieve long blood circulation by avoiding reticuloendothelial uptake from the blood. To date, many reports have described the use of PEGylated liposomes for long blood circulation. Woodle et al. reported the effect of various molecular weights (120, 750, 1,900, and 5,000) of PEG conjugated with distearoylphosphatidylethanolamine (DSPE)-modified liposomes on prolonged blood circulation [114]. They reported that longer forms of PEG with molecular weights of 1,900 and 5,000 kDa used to PEGylate liposomes with a size of about 100 nm prolonged their circulation in the blood. Maruyama et al. also reported a similar effect using large unilamellar liposomes with a size about 200 nm [115]. DOX-encapsulated liposomes showed enhanced tumor distribution through enhanced permeability and retention effects [116] and reduced adverse effects of DOX, especially cardiac toxicity [98, 117]. A DOX-encapsulated PEGylated liposome formulation (Doxil®) was approved by the US Food and Drug Administration in 1995. Recently, the development of Doxil® was reviewed by Barenholz et al. [98].

Although PEG modification of liposomes is a promising approach, the immune response against PEGylated liposomes should be considered for their therapeutic use.

Dams et al. reported that repeated injections of PEGylated liposomes significantly altered the pharmacokinetics of subsequently injected PEGylated liposomes [118]. A second dose of injected PEGylated liposomes was rapidly cleared from the blood. They studied the factors affecting the accelerated blood clearance of subsequently injected PEGylated liposomes [118]. Macrophage deletion experiments using clodronate-containing liposomes [120] provided evidence that Kupffer cells and spleen macrophages were involved in the reduced blood circulation. This effect did not depend on liposomal size but rather on lipid dose. In addition, they found that DOX-encapsulated PEGylated liposomes did not induce the enhanced clearance of a second injected “empty” PEGylated liposome. Ishida et al. also systemically investigated the accelerated clearance of subsequent PEGylated liposome administration [121-123] and demonstrated that PEGylated liposomes induced PEG-specific IgM that were responsible for the rapid elimination of second injected PEGylated liposomes [124, 125]. Furthermore, the induction of anti-PEG-specific IgM that recognized DOX-encapsulated PEGylated liposomes was significantly lower than that for “empty” PEGylated liposomes [126]. This observation partly explains the observation by Laveman et al. described above [119]. Such information about the immunological responses induced by PEGylated liposomes is important for the design of glycosylated liposomes.

5. Glycosylated liposomes for cell-selective targeting

Glycosylated liposomes have been studied for cell-selective delivery of drugs. It is important to consider the glycosylation methods used. Thus far, glycosylation of liposomes is achieved using glycoproteins or synthetic glycolipids attached to the liposomal surface. Compared with glycoproteins, it is easy to control synthetic glycolipids for targeting. Because glycosylated liposomes interact with endogenous components in blood, the hydrophobicity of synthetic glycolipids is important for stable incorporation *in vivo*. In addition, an adequate spacer length in synthetic glycolipids is required to display the ligands for recognition by receptors. Ligand modified PEGylated lipids have been used to display ligands on the surface of PEGylated liposomes because of a steric hindrance effect caused by PEG. However, PEG might be immunogenic. Thus, the design of glycosylated liposomes should be considered in a comprehensive way according to their application to disease. Fig. 2 summarizes the design of glycosylated liposomes by glycosylated lipids.

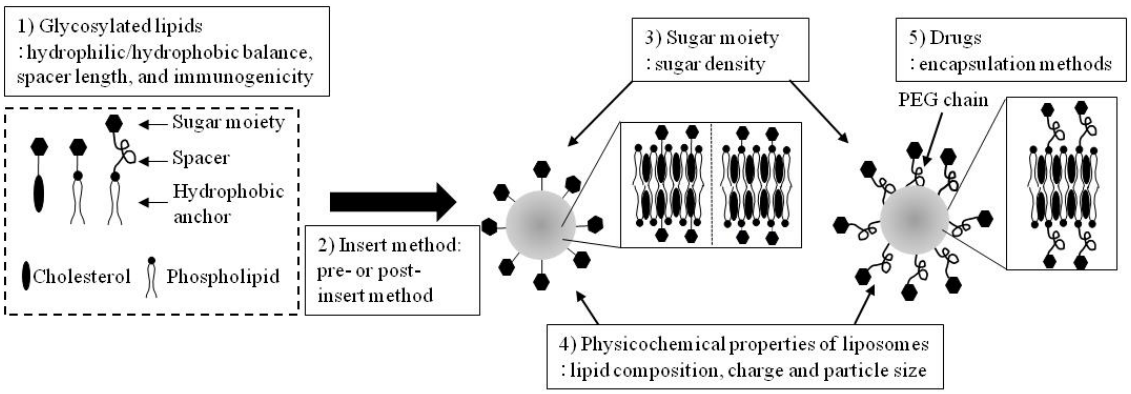

Fig. 2 Design of glycosylated liposomes by glycosylated lipids

Recently, there has been increasing attention paid to nucleic acid drugs and their application for refractory diseases. Various glycosylated liposomes have been developed for cell-selective delivery. However, both receptor recognition and release of nucleic acids from endosomes/lysosomes into the cytoplasm after internalization should be considered.

Table 2 summarizes an example of cell-selective targeting with liposomal carrier systems based on glycosylation. In this section, the use of glycosylated liposomes for cell-selective targeting of low-molecular-weight drugs and nucleic acids is reviewed.

Table 2 An example of glycosylation-mediated targeting of liposomal carriers

Target cells	Ligand	Administration route	Delivered compounds	Ref.
Liver PC	Asialofetuin	Intravenous injection	cholesterol	128
Liver PC	Asialofetuin	Intravenous injection	vitamin E, DiI	130
Liver NPC	(Tris) galactose	Intravenous injection	inulin	131
Liver PC	(Tris) galactose	Intravenous injection	cholesteroryl oleate	132
Liver PC	(Mono, bi, tris) galactose	Intravenous injection	inulin	135
Liver PC	Galactose	Intravenous injection	CHE	138, 139
Liver PC	Galactose	Intravenous injection	probucol	140
Liver PC	Galactose (PEG)	Intravenous injection	doxorubicin	143
Liver PC	Galactose	Intravenous injection	stavudin	136
Liver PC	Galactose	Intraportal injection	pDNA	156,160
Liver PC	Galactose	Intravenous injection	pDNA	168
Liver PC	Galactose	Intravenous injection	siRNA	171, 172, 173
Liver NPC	Mannose, Fucose	Intravenous injection	CHE	179
Liver NPC	Mannose	Intravenous injection	pDNA	192, 194
Liver NPC	Mannose	Intravenous injection	NFκB decoy	195
Liver NPC	Mannose (bubble)	Intravenous injection	siRNA	218
Peritoneal macrophages	(Oligo) mannose	Intraperitoneal injection	soluble leishmanial antigen	204
Peritoneal macrophages	(Oligo) mannose	Intraperitoneal injection	ovalbumin	205
DC	Mannose	Intravenous injection	mRNA	188
DC	Mannose (bubble)	Intravenous injection	pDNA	211
Alveolar macrophages	Mannose	Intratracheal injection	dexametasone palmitate	202
Alveolar macrophages	Mannose	Intratracheal injection	NFκB decoy	202
TAM	Mannose (bubble)	Intratumoral injection	NFκB decoy	219
Liver NPC	Fucose	Intravenous injection	NFκB decoy	225
Pancreatic cancer cells	Fucose	Intravenous injection	cisplatin	226
HSC	M6P	Intravenous injection	HSA-doxorubicin conjugate	227
HSC	M6P	Intravenous injection	DLPL	229

PC: parenchymal cells
NPC: non-parenchymal cells
DC: Dendritic cells
TAM: tumor associated macrophages
CHE: cholesteryl hexadecyl ether
DLPL: dilinoleoylphosphatidylcholine

5.1. Galactose modification

Similar to galactosylated macromolecular carriers, galactosylated liposomes have been studied as hepatocyte-selective targeting agents via asialoglycoprotein receptor-mediated uptake. To date, glycoproteins or synthetic glycolipids have been used to prepare galactosylated liposomes for targeted delivery.

5.1.1. Galactosylated liposomes

For efficient recognition by hepatocytes via asialoglycoprotein receptors, a rational design of galactosylated liposomes is required. Asialofetuin is a glycoprotein that possesses several tri-antennary galactose-terminated sugar chains, and is taken up by the

liver after intravenous administration in rats [127]. Tsuchiya et al. developed asialofetuin-modified liposomes for hepatocyte-selective targeting of drugs [128]. Asialofetuin was conjugated with palmitic acid, a hydrophobic anchor that fixes asialofetuin on to the liposomal surface. After intravenous administration, asialofetuin-modified liposomes were rapidly eliminated from the blood, and most were recovered from the liver [129]. Intrahepatic distribution studies revealed that asialofetuin-modified liposomes were selectively found in liver parenchymal cells [130] and Wu et al. reported that asialofetuin-modified liposomes distributed in the liver in combination with intravenously injected vitamin E improved the protective effect against CCl_4-induced acute liver injury in mice [130].

Synthetic glycolipids have been designed and studied for cell-selective targeting. Spanjer et al. reported that tri-antennary galactose-terminated cholesterol (tri-gal-chol) modified liposomes were efficiently distributed to the liver [131]. However, an intrahepatic distribution study showed that tri-gal-chol-modified liposomes were taken up by liver non-parenchymal cells (including Kupffer cells) via galactose/fucose recognizing receptors and not by liver parenchymal cells via the asialoglycoprotein receptors in rats. However, a rapid exchange of tri-gal-chol to endogenous components in the blood occurred because of the relatively high hydrophilicity of tri-gal-chol [132]. Sliedregt et al. synthesized a hydrophobic tri-gal-chol by adding one or more fatty acid chains to the glycolipid to increase their stable incorporation in galactosylated liposomes [132]. They found that 5% (mol) hydrophobic tri-gal-chol-modified galactosylated liposomes were taken up by asialoglycoprotein receptors on liver hepatocytes. In contrast, 50% (mol) hydrophobic tri-gal-chol-modified galactosylated liposomes were taken up by the galactose/fucose recognizing receptors on Kupffer cells in the liver. Sasaki and Murahashi et al. synthesized various synthetic galactosyl lipids to prepare galactosylated liposomes [133, 134] and found that galactose density on galactosylated liposomes was a critical factor for hepatocyte-selective targeting rather than the branching structure of the galactosylated lipid [135]. Recently, Garg et al. observed that uptake of galactosylated liposomes with a low galactosyl lipid content by parenchymal cells was significantly higher than for non-parenchymal cells [136]. This corresponded with a report by Sliedregt et al. [132]. These observations demonstrate the importance of the galactose density of galactosylated liposomes as well as the design of synthetic galactosylated lipids for hepatocyte-selective delivery.

For hepatocyte-selective targeting, our group synthesized cholesten-5-yloxy-N-(4-((1-imino-2-D-thiogalactosylethyl)amino)butyl)formamide (Gal-C4-Chol), a galactosylated cholesterol derivative, to prepare galactosylated

liposomes [137]. Introduction of many hydrophilic galactose moieties to a hydrophobic anchor might result in their removal by interaction with endogenous components in the blood. Therefore, one galactose moiety was conjugated to cholesterol via a spacer. After intravenous administration in mice, 5% (mol) Gal-C4-Chol-modified galactosylated liposomes were efficiently distributed to liver parenchymal cells [138]. The high uptake in liver parenchymal cells was observed the 3.5, 5.0, and 7.5% (mol) for Gal-C4-Chol-modified galactosylated liposomes [139]. To determine the effect of lipid composition in targeting, we prepared 5% (mol) Gal-C4-Chol-modified galactosylated liposomes involving different egg phosphatidylcholine or DSPC and cholesterol contents [140, 141]. Liver parenchymal cells selectivity was significantly affected by the lipid composition of galactosylated liposomes, suggesting that the lipid composition of galactosylated liposomes should be considered for cell-selective targeting.

To achieve a targeted and sustained drug delivery by galactosylated liposomes, we prepared polysorbate (Tween) 20 or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-polyethylene glycol (PEG(x)-DSPE) where x represents a mean molecular weight of PEG (2,000 or 350 kDa), which was added to galactosylated liposomes [142]. Incorporation of Tween 20 and PEG-DSPE reduced blood elimination and hepatic uptake. Furthermore, the incorporation of PEG_{350}-DSPE into galactosylated liposomes could control delivery to hepatocytes without losing its targeting capabilities. Recently, Wang et al. developed a novel cleavable PEG lipid, $PEG_{2,000}$-cholesteryl hemisuccinate ($PEG_{2,000}$-CHEMS), that can be metabolized by esterases in plasma and tissues, for targeted and sustained targeting into hepatocytes [143]. The incorporation of $PEG_{2,000}$-CHEMS into galactosylated liposomes directed the delivery of encapsulated DOX into hepatocytes without losing its targeting capability. DOX-encapsulated PEGylated and galactosylated liposomes exhibited the highest inhibitory effect in hepatocarcinoma 22-bearing mice. Therefore, a controlled and targeted method is an effective strategy to exert a high pharmacological effect of drugs used for therapy.

Regarding low-molecular-weight drug delivery, studies have reported the delivery of anti-cancer drugs to hepatocellular carcinomas (HCC). Xiao et al. synthesized a tetravalent galactosylated diethylenetriaminepenta-acetic acid-distearoyl phosphatidylethanolamine (4Gal-DTPA-DSPE) to prepare doxorubicin encapsulated galactosylated liposomes [144]. Either 5 or 10% (mol) of 4Gal-DTPA-DSPE was added to lipids to prepare galactosylated liposomes. Galactosylated liposomes had a prolonged blood retention and high liver accumulation after intravenous administration. Study of intrahepatic distribution demonstrated galactosylated liposomes selectively accumulated

in hepatocytes. As an example of applications for drug delivery, vitamin E [130], probucol [140], prostaglandin E_1 [145], DOX [146], retinoic acids [147], ara-C [148], N4-Octadecyl-1-β-D-arabinofuranosylcytosine [148], and stabudine [136, 149] have been used for hepatocyte-selective targeting. Because asialoglycoprotein receptors are expressed on normal hepatocytes, tumor selectivity is required for a rational cancer therapy. Our group developed a novel drug delivery system using enzymatic activity expressed specifically in tumors. It was reported that matrix metalloprotease-2 (MMP-2) plays a critical role in tumor progression, angiogenesis, and metastasis, and are overexpressed in HCC [150-153]. Using the enzymatic activity of MMP-2 could provide HCC-selective properties to galactosylated liposomes. Thus, our group developed a novel functional lipid, cleaved by the protease activity of MMP-2. The amino group of dioleoylphosphatidylethanolamine (DOPE) was conjugated [148] with PEGylated MMP-2 substrate peptide (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln), and MMP-2-cleavable PEG-peptide-DOPE (PEG-PD) was synthesized. PEG-PD incorporated galactosylated liposomes were taken up by human hematoma HepG2 cells in a dose-dependent manner and was dependent upon MMP-2 expression. Hatakeyama et al. also reported that a PEG-PD-modified multifunctional envelope-type nano device for tumor-selective delivery of pDNA and siRNA used the enzymatic degradation of the peptide by MMP-2 [153-155].

5.1.2. Nucleic acid delivery

Our group investigated the hepatocyte-selective delivery of nucleic acids using galactosylated cationic liposomes based on their physicochemical properties. Pharmacokinetic analysis of pDNA complexed with galactosylated PLL demonstrated that physicochemical properties such as charge and size of complexes should be optimized for efficient hepatocyte-selective targeting [32]. Therefore, we investigated the hepatocyte-selective delivery of pDNA using galactosylated cationic liposomes based on their physicochemical properties [156]. When pDNA was complexed with galactosylated cationic liposomes (galactosylated lipoplexes) via electrostatic interactions, a 5% glucose solution enabled the preparation of about 140-nm-sized galactosylated lipoplexes at a relatively high concentration for *in vivo* use. The cationic charge ratio (−:+) of galactosylated lipoplexes was from 1.0:2.3 to 1.0:3.1 and showed high gene expression in the liver. Furthermore, hepatocyte-selective gene expression was observed when lipoplexes were prepared at a charge ratio (−:+) of 1.0:2.3. In addition, galactosylation of the lipoplexes increased the tissue binding and internalization rate via asialoglycoprotein receptor-mediated endocytosis on hepatocytes

[157]. Letrou-Bonneval et al. reported that galactosylated multi-modular lipoplexes prepared at a charge ratio (−:+) of 1.0:2.0 showed asialoglycoprotein receptor-mediated transfection in primary cultured rat hepatocytes [158]. Penetration across the fenestrated sinusoidal endothelium by galactosylated lipoplexes might be limited. In addition, lipoplexes easily aggregate in higher salt concentrations [159]. Therefore, a reduction in size or improved stability of galactosylated lipoplexes in higher salt conditions would be a promising approach to enhance the transfection activity in hepatocytes. To solve this problem, we developed stabilized galactosylated lipoplexes by adding moderate amounts of sodium chloride during lipoplex formation based on our surface charge regulation (SCR) theory [160]. Galactosylated SCR lipoplexes had a mean diameter of 120 nm and had high stability in a high ionic solution (150 mM NaCl). The hepatic transfection activity of galactosylated SCR lipoplexes was about 10- to 20-fold higher than that of galactosylated lipoplexes. The pre-administration of excess galactosylated BSA inhibited hepatic gene expression in galactosylated SCR lipoplexes, suggesting the involvement of asialoglycoprotein receptor-mediated uptake on hepatocytes. Thus, controlling the physicochemical properties of galactosylated lipoplexes is important for the efficient hepatocyte-selective targeting of DNA.

Cationic-charged galactosylated lipoplexes interact with non-target cells or erythrocytes [161] via electrostatic interaction, therefore the use of PEGylated lipids would be a promising strategy to reduce such non-specific interactions. Perouzel et al. synthesized various glycosylated cholesterol derivatives to prepare glycosylated cationic liposomes, adenovirus core peptides, and pDNA ternary complexes [162]. Then, $PEG_{2,000}$-DSPE were incorporated into glycosylated ternary complexes by pre- or post-modification methods. Although a stabilizing effect was observed by the incorporation of $PEG_{2,000}$-DSPE (5-10% (mol)) in high salt conditions, transfection into HeLa cells was abolished using the same preparation conditions. Therefore, galactose might be required to conjugate the terminal of $PEG_{2,000}$ displayed on the liposomal surface for both efficient recognition by asialoglycoprotein receptors and the prevention of complex aggregation in higher salt conditions. As an alternate strategy, the use of degradable PEGylated lipids by either acid [163] or enzyme [148, 153] would be promising approach. Frisch et al. synthesized a novel galactosylated lipid. To display galactose moieties, a tri-antennary galactose moiety was attached to the distal end of the PEGylated lipids. The positive charge of the galactosylated-PEGylated lipoplexe was shielded by the PEG effect to some extent. Chloroquine inhibits endosomal maturation and can enhance the transfection activity of lipoplexes [164]. In the presence of chloroquine, targeted gene transfection was observed in human hepatoma HepG2 cells,

suggesting asialoglycoprotein receptor-mediated uptake. This result is in accord with those reported by our group using galactosylated lipoplexes [165]. These results also suggest that the pharmacokinetic process of endosomal escape is a rate-limiting process for efficient gene expression.

Considering these findings, both biodistribution and intracellular distribution should be controlled for efficient gene expression in hepatocytes. Therefore, a multifunctional carrier system by galactosylation should be developed. The use of a pH buffering effect could be an effective strategy to enhance transfection efficiency by promoting the release of drugs from the endosome to cytoplasm. Our group focused on histidine (His), an amino group with a pKa of 6.0. We synthesized a novel pH-sensitive histidine-modified galactosylated cholesterol derivative (Gal-His-C4-Chol), for efficient transfection to hepatocytes [166]. Galactosylated lipoplexes containing Gal-His-C4-Chol showed much greater transfection activity in HepG2 cells than conventional galactosylated lipoplexes and was dependent upon an asialoglycoprotein receptor-mediated mechanism. Recently, our group also demonstrated that lysine-histidine dendron-modified chitosan could improve the transfection efficiency of chitosan based on pH buffering capacity in HEK293 cells [167]. Recently, Hu et al. developed multifunctional galactosylated and PEGylated lipoplexes for the efficient nuclear delivery of pDNA in mice. In this multifunctional system, galactose, PEG, calcium phosphate, and monocyclic octa-arginine were introduced to control both biodistribution and intracellular distribution processes [168]. A high gene expression was observed in mouse hepatocytes. Therefore, both biodistribution and intracellular distribution should be considered as efficient strategies to obtain high gene expression in hepatocytes.

RNA interference (RNAi) can be induced by double-stranded siRNA, consisting of 21–25 nucleotides, which is incorporated into the RNAi-induced silencing complex and is a guide for cleavage of complementary target messenger RNA (mRNA) in the cytoplasm [169, 170]. An effective delivery system is essential for the application of siRNA as clinical treatment. Our previous study demonstrated that a siRNA (Ubc-13)/galactosylated cationic liposomes complex was efficiently delivered to hepatocytes [171]. Sonoke et al. also reported the hepatocyte-selective delivery of siRNA (firefly luciferase) using siRNA/galactosylated cationic liposomes complexes [172]. Regarding the use of these complexes for clinical use, Jiang et al. prevented liver ischemia reperfusion or concanavalin A-induced hepatitis via the hepatocyte-selective delivery of siRNA using siRNA/galactosylated cationic liposomes complexes [173, 174]. Thus, galactosylated cationic liposomes can deliver siRNA into hepatocytes.

5.2. Mannose modification

Similar to mannosylated macromolecular carriers, mannosylated liposomes have been studied for macrophage- and/or dendritic cell-selective targeting via mannose receptor-mediated uptake in various tissues. To date, mannosylated lipids have been used to prepare mannosylated liposomes for targeted delivery.

5.2.1. Development of mannosylated liposomes

Mannose-containing phospholipids from the cell wall of mycobacteria [175] and synthetic mannosylated lipids [176-191] have been used to prepare mannosylated liposomes for drug delivery or imaging of macrophages and/or dendritic cells. Mannosylated liposomes have been used for the treatment of diseases such as infections, inflammation, and cancer, etc..

For macrophage- and/or dendritic cell-selective targeting, our group synthesized cholesten-5-yloxy-N-(4-((1-imino-2-D-thiomannosylethyl)amino)butyl) formamide (Man-C4-Chol), a galactosylated cholesterol derivative, to prepare mannosylated liposomes [179]. To deliver drugs into macrophage- and/or dendritic cell in various tissues, we investigated various administration routes including intravenous [179, 192-197], intraperitoneal [198-201], and intratracheal [202, 203] administration. In addition, vaccination of peptides by subcutaneous [178] and intraperitoneal [204, 205] routes have been studied. Mannosylated liposomes were efficiently taken up by macrophages and/or dendritic cells in various tissues according to each administration route.

To obtain efficient recognition of complexes via mannose receptors expressed by macrophages, the rational design of mannosylated liposomes is required. Engel et al. synthesized alkylmannoside derivatives that possessed a hydrophobic anchor and hydrophilic head group containing a PEG spacer with increasing length between the hydrophilic head group and hydrophobic anchor moieties [182]. The effect of PEG as a spacer on macrophage uptake was investigated using mannosylated liposomes with alkylmannoside derivatives. Cellular uptake was increased by increasing PEG numbers of alkylmannoside derivatives. Thus, the spacer length of synthetic mannosylated lipids in mannosylated liposomes is important for recognition by mannose receptors on macrophages. Mannose density of mannosylated liposomes is also an important factor in the recognition of mannose receptors. Espuelas et al. synthesized multi-branched mannosylated lipids [186]. The dimannosylated ligands were recognized as efficiently as tetramannosylated lipids by mannose receptors expressed on immature human

dendritic cells. Man-C4-Chol contents in mannosylated liposomes enhanced the uptake of mannosylated liposomes from 2.5 to 7.5% (mol) by mannose receptor-mediated endocytosis in cultured rat alveolar macrophages [202]. This finding is in line with our previous report using mannosylated emulsion in mouse peritoneal macrophages [206]. Chono et al. showed that the uptake of bare liposomes by rat alveolar macrophages was dependent on particle size (100, 200, 400, 1,000, and 2,000 nm) and that mannosylation of liposomes with 4-aminophenyl-α-D-mannopyranoside enhanced uptake [185]. These results suggest the structure of mannosylated lipids, mannose density, and size of mannosylated liposomes should be optimized for efficient recognition by mannose receptors on macrophages and/or dendritic cells.

Examples of drug delivery or imaging applications by encapsulation of muramyl dipeptide [193], stavudine [184], dexamethasone palmitate [202], cytidine 5' diphosphocholine [187], amphotericin B [189], ^{64}Cu [191], and indocyanine green [206] have been reported. Similarly, encapsulation of various peptides to induce cytotoxic T lymphocytes for vaccination have been investigated [178, 204, 205].

5.2.2. Nucleic acid delivery

pDNA, NF-κB decoy, mRNA, and siRNA have been used for delivery to macrophages and/or dendritic cells. We investigated macrophage- and/or dendritic cell-selective delivery of nucleic acids using mannosylated cationic liposomes based on their physicochemical properties. Because it is not necessary to pass through fenestrae in the liver to reach Kupffer cells and liver endothelial cells, we prepared 200-nm-sized pDNA/mannosylated lipoplexes [192]. After intravenous administration in mice, the highest gene expression was observed in liver non-parenchymal cells including Kupffer cells and liver endothelial cells. A cationic charge ratio (−:+) of mannosylated lipoplexes from 1.0:2.3 to 1.0:3.1 demonstrated high gene expression in the liver [208]. When complexes were formed with higher charge ratios, transfection efficiency in the lung was highest, indicating non-specific interactions. These findings correspond with our previous reports of galactosylated lipoplexes *in vivo* and *in situ* [156, 157]. Consideration of the administration route is also important for pDNA delivery. After intravenous administration, many mannosylated lipoplexes were rapidly taken up by the liver [194]. Therefore, the intraperitoneal administration route was selected to avoid rapid uptake by Kupffer cells and achieve sustained gene expression in macrophages and dendritic cells, but not Kupffer cells [198]. Following intraperitoneal administration of mannosylated lipoplexes into mice, high gene expression was observed in macrophages and dendritic cells from peritoneally exuded cells and the spleen. This

supports a report by Ikehara et al. using ovalbumin-encapsulated oligomannose-coated liposomes via intraperitoneal administration [205]. Recently, Li et al. reported that mannosylated liposome/protamine/pDNA complexes exhibited a high transfection activity in bone marrow-derived dendritic cells [209] supporting the view that lipoplex mannosylation is an efficient strategy to obtain high transfection activity in macrophages and/or dendritic cells.

To control endosomal escape, we synthesized a histidine-modified mannosylated cholesterol derivative (Man-His-C4-Chol), for efficient transfection to macrophages [210]. Mannosylated lipoplexes containing Man-His-C4-Chol functioned similarly to galactosylated lipoplexes containing Gal-His-C4-Chol, and showed a higher transfection activity than conventional mannosylated lipoplexes, via a mannose receptor-mediated mechanism both *in vivo* and *in vitro*. These results are consistent with those recently reported by Perche et al. [188]. Therefore, both biodistribution and intracellular distribution could be useful strategies to obtain high gene expression in macrophages and/or dendritic cells.

The use of nucleic acids, DNA or mRNA vaccination for cytotoxic T lymphocyte induction [188, 199, 200, 211], CpG DND for cancer immunotherapy [196, 201], and NF-κB decoy delivery for anti-inflammatory therapy [H195, 203] has been reported.

5.2.3. Combination of bubble formulation with ultrasound exposure

Fig. 3 summarizes the combination method of glycosylated liposomes with physical stimuli for targeted drug delivery. Microbubbles can be destroyed by ultrasound exposure to generate microstreams or microjets. Consequently, transient holes are generated in cellular membranes. Therefore, the combined use of ultrasound exposure and microbubble formulation used as ultrasound contrast agents can enhance the transfection efficacy of naked pDNA by facilitating pDNA entry into the cells [212]. Suzuki et al. prepared ultrasound imaging gas-encapsulated liposomes (bubble liposomes) for naked pDNA mediated transfection in mice [213]. The bubble liposomes had a small size (about 446 nm) compared with conventional microbubble formulation [212]. We developed mannosylated bubble liposome/pDNA complexes (mannosylated bubble lipoplexes) for efficient cell-selective gene transfection with ultrasound exposure [214-217]. This system could be used for delivery of oligonucleotides such as siRNA or NF-κB decoy delivery into liver NPC [218] or tumor-associated macrophages [219]. Because cationic-charged bubble lipoplexes interact with erythrocytes, we developed ultrasound-responsive anionic-charged bubble lipopolyplexes as a platform of

glycosylation for safe transfection [220]. Therefore, optimization of physicochemical properties of mannosylated bubble formulations might be used for safe and efficient gene transfection into macrophages and/or dendritic cells. Another glycosylated bubble formulation, sialyl LewisX-modified microbubbles were previously developed for molecular imaging applications [221, 222].

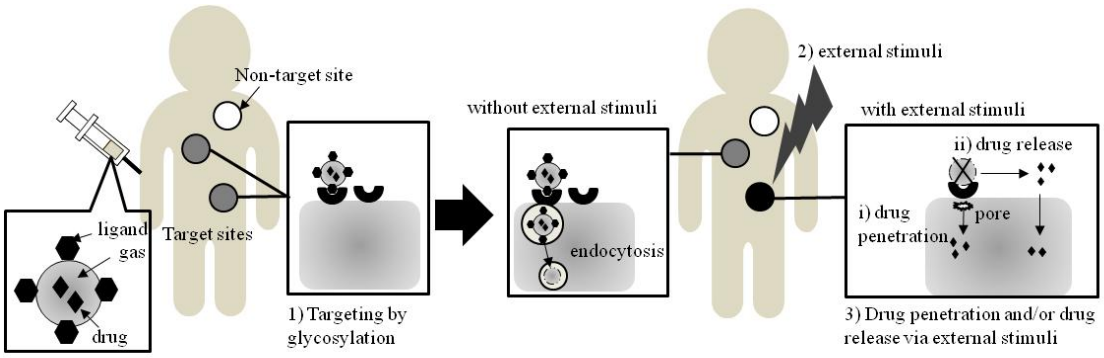

Fig. 3 Combination method of glycosylated liposomes with physical stimuli for targeted drug delivery

5.3. Fucose modification

Fucosylated liposomes have been studied as carriers for Kupffer cell or pancreatic cancer cell-selective delivery. Fucose rectin is found exclusively in hepatic Kupffer cells [223]. We synthesized cholesten-5-yloxy-N-(4-((1-imino-2-D-thiofucosylethyl)amino)butyl)formamide, a fucosylated cholesterol derivative, to prepare the fucosylated liposomes [179]. Intravenously injected fucosylated liposomes were rapidly taken up by the liver and were recovered from liver NPC. An *in vitro* uptake study using primary cultured rat sinusoidal endothelial cells and Kupffer cells demonstrated that fucosylated BSA was taken up by both sinusoidal cells and Kupffer cells [224]. The uptake of fucosylated BSA was significantly inhibited in the presence of excess mannosylated BSA and fucosylated BSA. In addition, fucosylated BSA was more Kupffer cell selective than mannosylated BSA. Thus, fucosylated liposomes might be more selective as Kupffer cell-targeting carriers.

Based on these findings, we investigated the use of fucosylated liposomes for NF-κB decoy delivery into Kupffer cells for the treatment of acute hepatitis [225]. NF-κB decoy was mainly recovered from liver NPC following the intravenous administration of fucosylated cationic liposomes/NF-κB decoy complexes (fucosylated complexes). In an acute hepatitis model, proinflammatory cytokines, alanine transaminase, and aspartate transaminase serum levels in LPS-infected mice were significantly attenuated by treatment with fucosylated complexes.

Recently, Yoshida et al. reported the pancreatic cancer cell-selective delivery of cisplatin using fucosylated liposomes by simultaneous injection of excess D-mannose (5 mg) to inhibit the hepatic uptake in tumor-bearing mice [226]. Under these experimental conditions, fucosylated liposomes selectively accumulated in pancreatic cancer cells and efficiently inhibited tumor growth as well as prolonging survival of tumor-bearing mice.

5.4. M6P modification

M6P/IGF II receptors are expressed on hepatic stellate cells [80-82] and M6P-modified liposomes have been studied as carriers for hepatic stellate cell-selective delivery. Kamps et al. conjugated M6P-HSA to the surface of liposomes for hepatic stellate cell-selective targeting [227-229]. In bile duct-ligated rats, M6P-HSA-modified liposomes selectively accumulated in hepatic stellate cells. Greupink et al. reported that M6P-HSA-DOX-conjugated modified liposomes inhibited hepatic stellate cell proliferation in bile duct-ligated rats. Similarly, Adrian et al. reported the use of M6P-HSA-modified liposomes or hemagglutinating virus of Japan liposomes for hepatic stellate cell-selective targeting [230, 231]. Therefore, M6P-modified liposomes might be an efficient carrier for hepatic stellate cell-selective delivery of drugs.

6. Future Perspectives

Although this review focused on glycosylated macromolecules and liposomes, glycosylated polymeric micelles would be a promising approach for targeted drug and gene delivery [232-235]. Kataoka et al. have reviewed the recent progress of the functional polymeric micelles for drug and gene delivery [236, 237].

For targeted drug delivery by glycosylation, both the glycosylation method and physicochemical properties should be optimized to achieve efficient cell-selective targeting by macromolecular and liposomal carriers. For nucleic acid delivery, the control of intracellular delivery is also required because of nucleic acid degradation by lysosomal enzymes. Therefore, introduction of functional groups that facilitate endosomal/lysosomal escape might be an effective strategy to improve transfection activity. Hossain and Akaike et al. reported that carbonate apatite/siRNA complex can facilitate the intracellular delivery of siRNA [238]; therefore the development of such a new carrier for glycosylation is important. To exploit the shielding effects of PEG, the use of biodegradable PEG cleaved by enzymes or low pH might provide a new targeting system. Moreover, the combined use of physical stimuli such as ultrasound exposure could enable enhanced targeting efficacy by glycosylated liposomes [239]. Because the use of ultrasound exposure is a promising approach, further optimization of the physicochemical properties and/or development of glycosylated lipids for glycosylated bubble formulations are required for more efficient and safe drug delivery systems.

References

[1] H. Sezaki, M. Hashida, Macromolecule-drug conjugates in targeted cancer chemotherapy, Crit. Rev. Ther. Drug Carr. Syst. 1 (1984) 1-38.

[2] Y. Takakura, M. Hashida, Macromolecular carrier systems for targeted drug delivery: pharmacokinetic considerations on biodistribution, Pharm. Res. 13 (1996) 820-831.

[3] W. Wijagkanalan, S. Kawakami, M. Hashida, Glycosylated carriers for cell-selective and nuclear delivery of nucleic acids, Front. Biosci. 16 (2011) 2970-2987.

[4] R.P. Harbottle, R.G. Cooper, S.L. Hart, A. Ladhoff, T. McKay, A.M. Knight, E. Wagner, A.D. Miller, C. Coutelle, An RGD-oligolysine peptide: a prototype construct for integrin-mediated gene delivery, Hum. Gene Ther. 9 (1998) 1037-1047.

[5] E. Kenjo, T. Asai, N. Yonenaga, H. Ando, T. Ishii, K. Hatanaka, K. Shimizu, Y. Urita, T. Dewa, M. Nango, H. Tsukada, N. Oku, Systemic delivery of small interfering RNA by use of targeted polycation liposomes for cancer therapy, Biol. Pharm. Bull. 36 (2012) 287-291.

[6] M. Vidal, J. Sainte-Marie, J.R. Philippot, A. Bienvenue, The influence of coupling transferrin to liposomes or minibeads on its uptake and fate in leukemic L2C cells, FEBS Lett. 216 (1987) 159-163.

[7] O. Ishida, K. Maruyama, H. Tanahashi, M. Iwatsuru, K. Sasaki, M. Eriguchi, H. Yanagie, Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo, Pharm. Res. 18 (2001) 1042-1048.

[8] A. Kikuchi, S. Sugaya, H. Ueda, K. Tanaka, Y. Aramaki, T. Hara, H. Arima, S. Tsuchiya, T. Fuwa, Efficient gene transfer to EGF receptor overexpressing cancer cells by means of EGF-labeled cationic liposomes, Biochem Biophys Res Commun. 227 (1996) 666-671.

[9] D. Kirpotin, J.W. Park, K. Hong, S. Zalipsky, W.L. Li, P. Carter, C.C. Benz, D. Papahadjopoulos, Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells in vitro, Biochemistry 36 (1997) 66-75.

[10] T. Miyano, W. Wijagkanalan, S. Kawakami, F. Yamashita, M. Hashida, Anionic amino acid dendrimer-trastuzumab conjugates for specific internalization in HER2-positive cancer cells, Mol. Pharm. 7 (2010) 1318-1327.

[11] Y. Higuchi, S. Kawakami, M. Hashida, Strategies for in vivo delivery of siRNAs: recent progress, BioDrugs. 24 (2010) 195-205.

[12] R. Kole, A.R. Krainer, S. Altman, RNA therapeutics: beyond RNA interference and

antisense oligonucleotides, Nat. Rev. Drug Discov. 11 (2012) 125-140.
[13] S.T. Crooke, R.S. Geary, Clinical pharmacological properties of mipomersen (Kynamro), a second generation antisense inhibitor of apolipoprotein B, Br. J. Clin. Pharmacol. 76 (2013) 269-276.
[14] Y. Zhang, Z. Wang, R.A. Gemeinhart, Progress in microRNA delivery, J. Control. Release, 172 (2013) 962-974.
[15] M. Kullberg, R. McCarthy, T.J. Anchordoquy, Systemic tumor-specific gene delivery, J. Control. Release, 172 (2013) 730-736.
[16] M. Yamada, M. Nishikawa, S. Kawakami, Y. Hattori, T. Nakano, F. Yamashita, M. Hashida, Tissue and intrahepatic distribution and subcellular localization of a mannosylated lipoplex after intravenous administration in mice, J. Control. Release, 98 (2004) 157-167.
[17] I.A. Khalil, K. Kogure, H. Akita, H. Harashima, Uptake pathways and subsequent intracellular trafficking in nonviral gene delivery, Pharmacol. Rev. 58 (2006) 32-45.
[18] Y. Takakura, R. I. Mahato, M. Hashida, Extravasation of macromolecules, Adv. Drug Deliv. Rev. 34 (1998) 93-108.
[19] F. Yamashita, M. Hashida, Pharmacokinetic considerations for targeted drug delivery, Adv. Drug Deliv. Rev. 65 (2013) 139-147.
[20] K. Mihara, M. Mori, T. Hojo, Y. Takakura, H. Sezaki, M. Hashida, Disposition characteristics of model macromolecules in the perfused rat kidney, Biol. Pharm. Bull. 16 (1993) 158-162.
[21] K. Nishida, K. Mihara, T. Takino, S. Nakane, Y. Takakura, M. Hashida, H. Sezaki, Hepatic disposition characteristics of electrically charged macromolecules in rat in vivo and in the perfused liver, Pharm. Res. 8 (1991) 437-444.
[22] S.F. Ma, M. Nishikawa, H. Katsumi, F. Yamashita, M. Hashida, Cationic charge-dependent hepatic delivery of amidated serum albumin, J. Control. Release. 102 (2005) 583-594.
[23] K. Kawabata, Y. Takakura, M. Hashida, The fate of plasmid DNA after intravenous injection in mice: involvement of scavenger receptors in its hepatic uptake, Pharm. Res. 12 (1995) 825-830.
[24] Y. Yamasaki, K. Sumimoto, M. Nishikawa, F. Yamashita, K. Yamaoka, M. Hashida, Y. Takakura, Pharmacokinetic analysis of in vivo disposition of succinylated proteins targeted to liver nonparenchymal cells via scavenger receptors: importance of molecular size and negative charge density for in vivo recognition by receptors, J. Pharmacol. Exp. Ther., 301 (2002) 467-477.
[25] Y. Yamasaki, J. Hisazumi, K. Yamaoka, Y. Takakura, Efficient scavenger

receptor-mediated hepatic targeting of proteins by introduction of negative charges on the proteins by aconitylation: the influence of charge density and size of the proteins molecules, Eur. J. Pharm. Sci., 18 (2003) 305-312.

[26] E. Wisse, An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids, J. Ultrastruct. Res., 31 (1970) 125-150.

[27] T. Daemen, M. Velinova, J. Regts, M. de Jager, R. Kalicharan, J. Donga, J.J. van der Want, G.L. Scherphof, Different intrahepatic distribution of phosphatidylglycerol and phosphatidylserine liposomes in the rat, Hepatology, 26 (1997) 416-423.

[28] M. Nishikawa, A. Kamijo, T. Fujita, Y. Takakura, H. Sezaki, M. Hashida, Synthesis and pharmacokinetics of a new liver-specific carrier, glycosylated carboxymethyl-dextran, and its application to drug targeting, Pharm. Res., 10 (1993) 1253-1261.

[29] L. Fiume, B. Bassi, C. Busi, A. Mattioli, G. Spinosa, H. Faulstich, Galactosylated poly(L-lysine) as a hepatotropic carrier of 9-β-D-arabinofuranosyladenine 5'-monophosphate, FEBS Lett., 203 (1986) 203-206.

[30] G.Y. Wu, C.H. Wu, Receptor-mediated gene delivery and expression in vivo, J. Biol. Chem., 263 (1988) 14621-14624.

[31] M. Hashida, S. Takemura, M. Nishikawa, Y. Takakura, Targeted delivery of plasmid DNA complexed with galactosylated poly(L-lysine), J. Control. Release, 53 (1998) 301-310.

[32] M. Nishikawa, S. Takemura, Y. Takakura, M. Hashida, Targeted delivery of plasmid DNA to hepatocytes in vivo: optimization of the pharmacokinetics of plasmid DNA/galactosylated poly(L-lysine) complexes by controlling their physicochemical properties, J. Pharmacol. Exp. Ther., 287 (1998) 408-415.

[33] S.J. Zheng, S. Zhong, J.J. Zhang, F. Chen, H. Ren, C.L. Deng, Distribution and anti-HBV effects of antisense oligodeoxynucleotides conjugated to galactosylated poly-L-lysine, World J. Gastroenterol., 9 (2003) 1251-1255.

[34] H. Hirabayashi, M. Nishikawa, Y. Takakura, M. Hashida, Development and pharmacokinetics of galactosylated poly-L-glutamic acid as a biodegradable carrier for liver-specific drug delivery, Pharm. Res., 13 (1996) 880-884.

[35] K. Akamatsu, Y. Yamasaki, M. Nishikawa, Y. Takakura, M. Hashida, Development of a hepatocyte-specific prostaglandin E_1 polymeric prodrug and its potential for preventing carbon tetrachloride-induced fulminant hepatitis in mice, J. Pharmacol. Exp. Ther., 290 (1999) 1242-1249.

[36] M. Hashida, K. Akamatsu, M. Nishikawa, F. Yamashita, Y. Takakura, Design of

polymeric prodrugs of prostaglandin E_1 having galactose residue for hepatocyte targeting, J. Control. Release, 62 (1999) 253-262.

[37] M.A. Zanta, O. Boussif, A. Adib, J.P. Behr, In vitro gene delivery to hepatocytes with galactosylated polyethylenimine, Bioconjug. Chem., 8 (1997) 839-844.

[38] T. Bettinger, J.S. Remy, P. Erbacher, Size reduction of galactosylated PEI/DNA complexes improves lectin-mediated gene transfer into hepatocytes, Bioconjug. Chem., 10 (1999) 558-561.

[39] K. Sagara, S.W. Kim, A new synthesis of galactose-poly(ethylene glycol)-polyethylenimine for gene delivery to hepatocytes, J. Control. Release, 79 (2002) 271-281.

[40] K. Morimoto, M. Nishikawa, S. Kawakami, T. Nakano, Y. Hattori, S. Fumoto, F. Yamashita, M. Hashida, Molecular weight-dependent gene transfection activity of unmodified and galactosylated polyethyleneimine on hepatoma cells and mouse liver, Mol. Ther., 7 (2003) 254-261.

[41] S. Fumoto, S. Kawakami, M. Ishizuka, M. Nishikawa, F. Yamashita, M. Hashida, Analysis of hepatic disposition of unmodified and galactosylated polyethylenimine complexed with plasmid DNA in the rat perfused liver, Drug Metab. Pharmacokinet., 18 (2003) 230-237.

[42] K. Ma, H. Shen, S. Shen, M. Xie, C. Mao, L. Qiu, Y. Jin, Development of a successive targeting liposome with multi-ligand for efficient targeting gene delivery, J. Gene Med., 13 (2011) 290-301.

[43] I.K. Park, Y.H. Park, B.A. Shin, E.S. Choi, Y.R. Kim, T. Akaike, C.S. Cho, Galactosylated chitosan-graft-dextran as hepatocyte-targeting DNA carrier, J. Control. Release, 69 (2000) 97-108.

[44] I.K. Park, T.H. Kim, Y.H. Park, B.A. Shin, E.S. Choi, E.H. Chowdhury, T. Akaike, C.S. Cho, Galactosylated chitosan-graft-poly(ethylene glycol) as hepatocyte-targeting DNA carrier, J. Control. Release, 76 (2001) 349-362.

[45] S. Gao, J. Chen, X. Xu, Z. Ding, Y.H. Yang, Z. Hua, J. Zhang, Galactosylated low molecular weight chitosan as DNA carrier for hepatocyte-targeting, Int. J. Pharm., 255 (2003) 57-68.

[46] T.H. Kim, I.K. Park, J.W. Nah, Y.J. Choi, C.S. Cho, Galactosylated chitosan/DNA nanoparticles prepared using water-soluble chitosan as a gene carrier, Biomaterials, 25 (2004) 3783-3792.

[47] M. Cheng, Q. Li, T. Wan, X. Hong, H. Chen, B. He, Z. Cheng, H. Xu, T. Ye, B. Zha, J. Wu, R. Zhou, Synthesis and efficient hepatocyte targeting of galactosylated chitosan as a gene carrier in vitro and in vivo, J. Biomed. Mater. Res. B Appl.

Biomater., 99 (2011) 70-80.
[48] K. Wada, H. Arima, T. Tsutsumi, F. Hirayama, K. Uekama, Enhancing effects of galactosylated dendrimer/α-cyclodextrin conjugates on gene transfer efficiency, Biol. Pharm. Bull., 28 (2005) 500-505.
[49] B. Lu, D.Q. Wu, H. Zheng, C.Y. Quan, X.Z. Zhang, R.X. Zhuo, Galactosyl conjugated N-succinyl-chitosan-graft-polyethylenimine for targeting gene transfer, Mol. Biosyst., 6 (2010) 2529-2538.
[50] J.H. Kim, Y.K. Kim, M.T. Arash, S.H. Hong, J.H. Lee, B.N. Kang, Y.B. Bang, C.S. Cho, D.Y. Yu, H.L. Jiang, M.H. Cho, Galactosylation of chitosan-graft-spermine as a gene carrier for hepatocyte targeting in vitro and in vivo, J. Nanosci. Nanotechnol., 12 (2012) 5178-51784.
[51] K. Akamatsu, M. Imai, Y. Yamasaki, M. Nishikawa, Y. Takakura, M. Hashida, Disposition characteristics of glycosylated poly(amino acids) as liver cell-specific drug carrier, J. Drug Target., 6 (1998), 229-239.
[52] M. Nishikawa, C. Miyazaki, F. Yamashita, Y. Takakura, M. Hashida, Galactosylated proteins are recognized by the liver according to the surface density of galactose moieties, Am. J. Physiol., 268 (1995) G849-856.
[53] O. Boussif, F. Lezoualc'h, M.A. Zanta, M.D. Mergny, D. Scherman, B. Demeneix, J.P. Behr, A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine, Proc. Natl. Acad. Sci. USA., 92 (1995) 7297-7301.
[54] M. Nishikawa, M. Yamauchi, K. Morimoto, E. Ishida, Y. Takakura, M. Hashida, Hepatocyte-targeted in vivo gene expression by intravenous injection of plasmid DNA complexed with synthetic multi-functional gene delivery system, Gene Ther., 7 (2000) 548-555.
[55] T.H. Kim, S.I. Kim, T. Akaike, C.S. Cho, Synergistic effect of poly(ethylenimine) on the transfection efficiency of galactosylated chitosan/DNA complexes, J. Control. Release, 105 (2005) 354-366.
[56] M. Hohokabe, Y. Higuchi, H. Mukai, S. Kawakami, M. Hashida, Hepatocyte-selective gene transfer by galactosylated protein/linear polyethyleneimine/plasmid DNA complexes in mice, J Biomed Nanotech, 3 (2007) 277-284.
[57] J.C. Robbins, M.H. Lam, C.S. Tripp, R.L. Bugianesi, M.M. Ponpipom, T.Y. Shen, Synthetic glycopeptide substrates for receptor-mediated endocytosis by macrophages, Proc. Natl. Acad. Sci. USA., 78 (1981) 7294-7298.
[58] C. Tietze, P. Schlesinger, P. Stahl, Mannose-specific endocytosis receptor of

alveolar macrophages: demonstration of two functionally distinct intracellular pools of receptor and their roles in receptor recycling, J. Cell Biol., 92 (1982) 417-424.

[59] S.A. Weston, C.R. Parish, Evidence that mannose recognition by splenic sinusoidal cells plays a role in the splenic entry of lymphocytes, Eur. J. Immunol., 22 (1992) 1975-1981.

[60] F. Sallusto, M. Cella, C. Danieli, A. Lanzavecchia, Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products, J. Exp. Med., 182 (1995) 389-400.

[61] W. Wijagkanalan, S. Kawakami, M. Hashida, Glycosylated carriers for cell-selective and nuclear delivery of nucleic acids, Front. Biosci. 16 (2011) 2970-2987.

[62] P. Erbacher, M.T. Bousser, J. Raimond, M. Monsigny, P. Midoux, A.C. Roche, Gene transfer by DNA/glycosylated polylysine complexes into human blood monocyte-derived macrophages, Hum. Gene Ther., 7 (1996) 721-729.

[63] R.I. Mahato, S. Takemura, K. Akamatsu, M. Nishikawa, Y. Takakura, M.Hashida, Physicochemical and disposition characteristics of antisense oligonucleotides complexed with glycosylated poly(L-lysine), Biochem. Pharmacol., 53 (1997) 887-895.

[64] M. Nishikawa, S. Takemura, F. Yamashita, Y. Takakura, D.K. Meijer, M. Hashida, P.J. Swart, Pharmacokinetics and in vivo gene transfer of plasmid DNA complexed with mannosylated poly(L-lysine) in mice, J. Drug Target., 8 (2000) 29-38.

[65] N. Brandhonneur, F. Chevanne, V. Vié, B. Frisch, R. Primault, M.F. Le Potier, P. Le Corre, Specific and non-specific phagocytosis of ligand-grafted PLGA microspheres by macrophages, Eur. J. Pharm. Sci., 36 (2009) 474-485.

[66] S.S. Diebold, H. Lehrmann, M. Kursa, E. Wagner, M. Cotten, M. Zenke, Efficient gene delivery into human dendritic cells by adenovirus polyethylenimine and mannose polyethylenimine transfection, Hum. Gene Ther., 10 (1999) 775-786.

[67] S.S. Diebold, M. Kursa, E. Wagner, M. Cotten, M. Zenke, Mannose polyethylenimine conjugates for targeted DNA delivery into dendritic cells, J. Biol. Chem., 274 (1999) 19087-19094.

[68] I.Y. Park, I.Y. Kim, M.K. Yoo, Y.J. Choi, M.H. Cho, CS. Cho, Mannosylated polyethylenimine coupled mesoporous silica nanoparticles for receptor-mediated gene delivery, Int. J. Pharm., 359 (2008) 280-287.

[69] N. Kim, D. Jiang, A.M. Jacobi, K.A. Lennox, S.D. Rose, M.A. Behlke, A.K.Salem, Synthesis and characterization of mannosylated pegylated polyethylenimine as a

carrier for siRNA, Int. J. Pharm., 427 (2012) 123-133.

[70] X. Sun, S. Chen, J. Han, Z. Zhang, Mannosylated biodegradable polyethyleneimine for targeted DNA delivery to dendritic cells, Int. J. Nanomedicine, 7 (2012) 2929-2942.

[71] M. Hashimoto, M. Morimoto, H. Saimoto, Y. Shigemasa, H. Yanagie, M. Eriguchi, T. Sato, Gene transfer by DNA/mannosylated chitosan complexes into mouse peritoneal macrophages, Biotechnol. Lett. 28 (2006) 815-821.

[72] T.H. Kim, H. Jin, H.W. Kim, M.H. Cho, C.S. Cho, Mannosylated chitosan nanoparticle-based cytokine gene therapy suppressed cancer growth in BALB/c mice bearing CT-26 carcinoma cells, Mol. Cancer Ther., 5 (2006) 1723-1732.

[73] W. Yao, Y. Peng, M. Du, J. Luo, L. Zong, Preventative vaccine-loaded mannosylated chitosan nanoparticles intended for nasal mucosal delivery enhance immune responses and potent tumor immunity, Mol. Pharm., 10 (2013) 2904-2914.

[74] K. Wada, H. Arima, T. Tsutsumi, Y. Chihara, K. Hattori, F. Hirayama, K. Uekama, Improvement of gene delivery mediated by mannosylated dendrimer/α-cyclodextrin conjugates, J. Control. Release, 104 (2005) 397-413.

[75] H. Arima, Y. Chihara, M. Arizono, S. Yamashita, K. Wada, F. Hirayama, K. Uekama, Enhancement of gene transfer activity mediated by mannosylated dendrimer/α -cyclodextrin conjugate (generation 3, G3), J. Control. Release, 116 (2006) 64-74.

[76] P. Opanasopit, K. Shirashi, M. Nishikawa, F. Yamashita, Y. Takakura, M. Hashida, In vivo recognition of mannosylated proteins by hepatic mannose receptors and mannan-binding protein, Am. J. Physiol. Gastrointest. Liver Physiol., 280 (2001) G879-889.

[77] P. Ghosh, N.M. Dahms, S. Kornfeld, Mannose 6-phosphate receptors: new twists in the tale, Nat. Rev. Mol. Cell Biol., 4 (2003) 202-212.

[78] P. Lemansky, I. Fester, E. Smolenova, C. Uhländer, A. Hasilik, The cation-independent mannose 6-phosphate receptor is involved in lysosomal delivery of serglycin, J. Leukoc. Biol., 81 (2007) 1149-1158.

[79] I. Martin-Kleiner, K.G. Troselj, Mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) in carcinogenesis, Cancer Lett., 289 (2010) 11-22.

[80] P.J. de Bleser, P. Jannes P, S.C. van Buul-Offers, C.M. Hoogerbrugge, C.F. van Schravendijk, T. Niki, V. Rogiers, J.L. van den Brande, E. Wisse, A. Geerts, Insulinlike growth factor-II/mannose 6-phosphate receptor is expressed on CCl_4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-β in cocultures with sinusoidal endothelial cells, Hepatology, 21

(1995) 1429-1437.

[81] P.J. De Bleser, C.D. Scott, T. Niki, G. Xu, E. Wisse, A. Geerts, Insulin-like growth factor II/mannose 6-phosphate-receptor expression in liver and serum during acute CCl_4 intoxication in the rat, Hepatology, 23 (1996) 1530-1537.

[82] J.A. Weiner, A. Chen, B.H. Davis, E-box-binding repressor is down-regulated in hepatic stellate cells during up-regulation of mannose 6-phosphate/insulin-like growth factor-II receptor expression in early hepatic fibrogenesis, J. Biol. Chem., 273 (1998) 15913-15919.

[83] A. Geerts, History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells, Semin. Liver Dis., 21 (2001) 311-335.

[84] L. Beljaars, G. Molema, B. Weert, H. Bonnema, P. Olinga, G.M. Groothuis, D.K. Meijer, K. Poelstra, Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells, Hepatology, 29 (1999) 1486-1493.

[85] L. Beljaars, P. Olinga, G. Molema, P. de Bleser, A. Geerts, G.M. Groothuis, D.K. Meijer, K. Poelstra, Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin ($M6P_{28}$-HSA), Liver, 21 (2001) 320-328.

[86] R. Greupink, H.I. Bakker, W. Bouma, C. Reker-Smit, D.K. Meijer, L. Beljaars, K. Poelstra, The antiproliferative drug doxorubicin inhibits liver fibrosis in bile duct-ligated rats and can be selectively delivered to hepatic stellate cells in vivo, J. Pharmacol. Exp. Ther., 317 (2006) 514-521.

[87] W.I. Hagens, L. Beljaars, D.A. Mann, M.C. Wright, B. Julien, S. Lotersztajn, C. Reker-Smit, K. Poelstra, Cellular targeting of the apoptosis-inducing compound gliotoxin to fibrotic rat livers, J. Pharmacol. Exp. Ther., 324 (2008) 902-910.

[88] T. Gonzalo, L. Beljaars, M. van de Bovenkamp, K. Temming, A.M. van Loenen, C. Reker-Smit, D.K. Meijer, M. Lacombe, F. Opdam, G. Kéri, L. Orfi, K. Poelstra, R.J. Kok, Local inhibition of liver fibrosis by specific delivery of a platelet-derived growth factor kinase inhibitor to hepatic stellate cells, J. Pharmacol. Exp. Ther., 321 (2007) 856-865.

[89] M. Moreno, T. Gonzalo, R.J. Kok, P. Sancho-Bru, M. van Beuge, J. Swart, J. Prakash, K. Temming, C. Fondevila, L. Beljaars, M. Lacombe, P. van der Hoeven, V. Arroyo, K. Poelstra, D.A. Brenner, P. Ginès, R. Bataller, Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats, Hepatology, 51 (2010) 942-952.

[90] M.M. van Beuge, J. Prakash, M. Lacombe, E. Post, C. Reker-Smit, L. Beljaars, K. Poelstra, Increased liver uptake and reduced hepatic stellate cell activation with a

cell-specific conjugate of the Rho-kinase inhibitor Y27632, Pharm. Res., 28 (2011) 2045-2054.

[91] M.M. van Beuge, J. Prakash, M. Lacombe, R. Gosens, E. Post, C. Reker-Smit, L. Beljaars, K. Poelstra, Reduction of fibrogenesis by selective delivery of a Rho kinase inhibitor to hepatic stellate cells in mice, J. Pharmacol. Exp. Ther., 337 (2011) 628-635.

[92] S. Klein, M.M. Van Beuge, M. Granzow, L. Beljaars, R. Schierwagen, S. Kilic, I. Heidari, S. Huss, T. Sauerbruch, K. Poelstra, J. Trebicka, HSC-specific inhibition of Rho-kinase reduces portal pressure in cirrhotic rats without major systemic effects, J. Hepatol., 57 (2012) 1220-1227.

[93] M.M. van Beuge, J. Prakash, M. Lacombe, E. Post, C. Reker-Smit, L. Beljaars, K. Poelstra, Enhanced effectivity of an ALK5-inhibitor after cell-specific delivery to hepatic stellate cells in mice with liver injury, PLoS One, 8 (2013) e56442.

[94] J. Prakash, L. Beljaars, A.K. Harapanahalli, M. Zeinstra-Smith, A. de Jager-Krikken, M. Hessing, H. Steen, K. Poelstra, Tumor-targeted intracellular delivery of anticancer drugs through the mannose-6-phosphate/insulin-like growth factor II receptor, Int. J. Cancer, 126 (2010) 1966-1981.

[95] Z. Ye, K. Cheng, R.V. Guntaka, R.I. Mahato, Targeted delivery of a triplex-forming oligonucleotide to hepatic stellate cells, Biochemistry, 44 (2005) 4466-4476.

[96] Z. Ye, K. Cheng, R.V. Guntaka, R.I. Mahato, Receptor-mediated hepatic uptake of M6P-BSA-conjugated triplex-forming oligonucleotides in rats, Bioconjug. Chem., 17 (2006) 823-830.

[97] L. Zhu, R.I. Mahato, Targeted delivery of siRNA to hepatocytes and hepatic stellate cells by bioconjugation, Bioconjug. Chem., 21 (2010) 2119-2127.

[98] Y. Barenholz, Doxil®-the first FDA-approved nano-drug: lessons learned, J. Control. Release, 160 (2012) 117-134.

[99] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, Adv. Drug Deliv. Rev., 65 (2013) 36-48.

[100] H. Ishihara, Current status and prospects of polyethyleneglycol-modified medicines, Biol. Pharm. Bull., 36 (2013) 883-888.

[101]T.M. Allen, C.B. Hansen, D.E.L. de Menezes, Pharmacokinetics of long-circulating liposomes, Adv. Drug Deliv. Rev., 16 (1995) 267-284.

[102] J. Dave, HM. Patel, Differentiation in hepatic and splenic phagocytic activity during reticuloendothelial blockade with cholesterol-free and cholesterol-rich liposomes, Biochim. Biophys. Acta, 888 (1986) 184-190.

[103] S.C. Semple, A. Chonn, P.R. Cullis, Influence of cholesterol on the association of

plasma proteins with liposomes, Biochemistry, 35 (1996) 2521-2525.
[104] R.L. Juliano, D. Stamp, The effect of particle size and charge on the clearance rates of liposomes and liposome encapsulated drugs, Biochem. Biophys. Res. Commun., 63 (1975) 651-658.
[105] T.M. Allen, P. Williamson, R.A. Schlegel, Phosphatidylserine as a determinant of reticuloendothelial recognition of liposome models of the erythrocyte surface, Proc. Natl. Acad. Sci. USA, 85 (1988) 8067-8071.
[106] A. Gabizon, D. Papahadjopoulos, The role of surface charge and hydrophilic groups on liposome clearance in vivo, Biochim. Biophys. Acta, 1103 (1992) 94-100.
[107] F. Liu, H. Qi, L. Huang, D. Liu, Factors controlling the efficiency of cationic lipid-mediated transfection in vivo via intravenous administration, Gene Ther., 4 (1997) 517-523.
[108] S. Li, L. Huang, In vivo gene transfer via intravenous administration of cationic lipid-protamine-DNA (LPD) complexes, Gene Ther., 4 (1997) 891-900.
[109] P. Charoensit, S. Kawakami, Y. Higuchi, F. Yamashita, M. Hashida, Enhanced growth inhibition of metastatic lung tumors by intravenous injection of ATRA-cationic liposome/IL-12 pDNA complexes in mice, Cancer Gene Ther., 17 (2010) 512-522.
[110] W. Yeeprae, S. Kawakami, S. Suzuki, F. Yamashita, M. Hashida, Physicochemical and pharmacokinetic characteristics of cationic liposomes, Pharmazie, 61 (2006) 102-105.
[111] T.M. Allen, A. Chonn, Large unilamellar liposomes with low uptake into the reticuloendothelial system, FEBS Lett., 223 (1987) 42-46.
[112] A.L. Klibanov, K. Maruyama, V.P. Torchilin, L. Huang, Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes, FEBS Lett., 268 (1990) 235-237.
[113] T.M. Allen, C. Hansen, F. Martin, C. Redemann, A. Yau-Young, Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives in vivo, Biochim. Biophys. Acta, 1066 (1991) 29-36.
[114] M.C. Woodle, K.K. Matthay, M.S. Newman, J.E. Hidayat, L.R. Collins, C. Redemann, F.J. Martin, D. Papahadjopoulos, Versatility in lipid compositions showing prolonged circulation with sterically stabilized liposomes, Biochim. Biophys. Acta, 1105 (1992) 193-200.
[115] K. Maruyama, T. Yuda, A. Okamoto, S. Kojima, A. Suginaka, M. Iwatsuru, Prolonged circulation time in vivo of large unilamellar liposomes composed of

distearoyl phosphatidylcholine and cholesterol containing amphipathic poly(ethylene glycol), Biochim. Biophy.s Acta, 1128 (1992) 44-49.

[116] H. Maeda, Macromolecular therapeutics in cancer treatment: the EPR effect and beyond, J. Control. Release, 164 (2012) 138-144.

[117] A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, F. Martin, A. Huang, Y. Barenholz, Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes, Cancer Res., 54 (1994) 987-992.

[118] E.T. Dams, P. Laverman, W.J. Oyen, G. Storm, G.L. Scherphof, J.W. van Der Meer, F.H. Corstens, O.C.Boerman, Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes, J. Pharmacol. Exp. Ther., 292 (2000) 1071-1079.

[119] P. Laverman, M.G. Carstens, O.C. Boerman, E.T. Dams, W.J. Oyen, N. van Rooijen, F.H. Corstens, G. Storm, Factors affecting the accelerated blood clearance of polyethylene glycol-liposomes upon repeated injection, J. Pharmacol. Exp. Ther., 298 (2001) 607-612.

[120] N. Van Rooijen, A. Sanders, Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications, J. Immunol. Methods, 174 (1994) 83-93.

[121] T. Ishida, R. Maeda, M. Ichihara, K. Irimura, H. Kiwada, Accelerated clearance of PEGylated liposomes in rats after repeated injections, J. Control. Release, 88 (2003) 35-42.

[122] T. Ishida, K. Masuda, T. Ichikawa, M. Ichihara, K. Irimura, H. Kiwada, Accelerated clearance of a second injection of PEGylated liposomes in mice, Int. J. Pharm., 255 (2003) 167-174.

[123] T. Ishida, T. Ichikawa, M. Ichihara, Y. Sadzuka, H. Kiwada, Effect of the physicochemical properties of initially injected liposomes on the clearance of subsequently injected PEGylated liposomes in mice. J. Control. Release, 95 (2004) 403-412.

[124] T. Ishida, M. Ichihara, X. Wang, K. Yamamoto, J. Kimura, E. Majima, H. Kiwada, Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes, J. Control. Release, 112 (2006) 15-25.

[125] T. Ishida, H. Kiwada, Anti-polyethyleneglycol antibody response to PEGylated substances, Biol. Pharm. Bull., 36 (2013) 889-891.

[126] T. Ishida, K. Atobe, X. Wang, H. Kiwada, Accelerated blood clearance of

PEGylated liposomes upon repeated injections: effect of doxorubicin-encapsulation and high-dose first injection, J. Control. Release, 115 (2006) 251-258.

[127] E. Regoeczi, M.T. Debanne, M.C. Hatton, A. Koj, Elimination of asialofetuin and asialoorosomucoid by the intact rat. Quantitative aspects of the hepatic clearance mechanism, Biochim. Biophys. Acta, 541 (1978) 372-384.

[128] S. Tsuchiya, Y. Aramaki, T. Hara, K. Hosoi, A. Okada, Preparation and disposition of asialofetuin-labelled liposome, Biopharm. Drug Dispos., 7 (1986) 549-558.

[129] T. Hara, Y. Aramaki, S. Tsuchiya, K. Hosoi, A. Okada, Specific incorporation of asialofetuin-labeled liposomes into hepatocytes through the action of galactose-binding protein, Biopharm. Drug Dispos., 8 (1987) 327-339.

[130] J. Wu, P. Liu, J.L. Zhu, S. Maddukuri, M.A. Zern, Increased liver uptake of liposomes and improved targeting efficacy by labeling with asialofetuin in rodents, Hepatology, 27 (1998) 772-778.

[131] H.H. Spanjer, T.J. van Berkel, G.L. Scherphof, H.J.Kempen, The effect of a water-soluble tris-galactoside terminated cholesterol derivative on the in vivo fate of small unilamellar vesicles in rats, Biochim. Biophys. Acta, 816 (1985) 396-402.

[132] L.A. Sliedregt, P.C. Rensen, E.T. Rump, P.J. van Santbrink, M.K. Bijsterbosch, A.R. Valentijn, G.A. van der Marel, J.H. van Boom, T.J. van Berkel, E.A. Biessen, Design and synthesis of novel amphiphilic dendritic galactosides for selective targeting of liposomes to the hepatic asialoglycoprotein receptor, J. Med. Chem., 42 (1999) 609-618.

[133] A. Sasaki, N. Murahashi, H. Yamada, A. Morikawa, Syntheses of novel galactosyl ligands for liposomes and their accumulation in the rat liver, Biol. Pharm. Bull., 17 (1994) 680-685.

[134] A. Sasaki, N. Murahashi, H. Yamada, A. Morikawa, Syntheses of novel galactosyl ligands for liposomes and the influence of the spacer on accumulation in the rat liver, Biol. Pharm. Bull., 18 (1995) 740-746.

[135] N. Murahashi, H. Ishihara, A. Sasaki, M. Sakagami, H. Hamana, Hepatic accumulation of glutamic acid branched neogalactosyllipid modified liposomes, Biol. Pharm. Bull., 20 (1997) 259-266.

[136] M. Garg, T. Dutta, N.K. Jain, Reduced hepatic toxicity, enhanced cellular uptake and altered pharmacokinetics of stavudine loaded galactosylated liposomes, Eur. J. Pharm. Biopharm., 67 (2007) 76-85.

[137] S. Kawakami, F. Yamashita, M. Nishikawa, Y. Takakura, M. Hashida, Asialoglycoprotein receptor-mediated gene transfer using novel galactosylated cationic liposomes, Biochem. Biophys. Res. Commun., 252 (1998) 78-83.

[138] S. Kawakami, C. Munakata, S. Fumoto, F. Yamashita, M. Hashida, Novel galactosylated liposomes for hepatocyte-selective targeting of lipophilic drugs, J. Pharm. Sci., 90 (2001) 105-113.

[139] C. Managit, S. Kawakami, F. Yamashita, M. Hashida, Effect of galactose density on asialoglycoprotein receptor-mediated uptake of galactosylated liposomes, J. Pharm. Sci., 94 (2005) 2266-2275.

[140] Y. Hattori, S. Kawakami, F. Yamashita, M. Hashida, Controlled biodistribution of galactosylated liposomes and incorporated probucol in hepatocyte-selective drug targeting, J. Control. Release, 69 (2000) 369-377.

[141] A. Murao, M. Nishikawa, C. Managit, J. Wong, S. Kawakami, F. Yamashita, M. Hashida, Targeting efficiency of galactosylated liposomes to hepatocytes in vivo: effect of lipid composition, Pharm. Res., 19 (2002) 1808-1814.

[142] C. Managit, S. Kawakami, M. Nishikawa, F. Yamashita, M. Hashida, Targeted and sustained drug delivery using PEGylated galactosylated liposomes, Int. J. Pharm., 266 (2003) 77-84.

[143] S. Wang, H. Xu, J. Xu, Y. Zhang, Y. Liu, Y.H. Deng, D. Chen, Sustained liver targeting and improved antiproliferative effect of doxorubicin liposomes modified with galactosylated lipid and PEG-lipid, AAPS PharmSciTech., 11 (2010) 870-877.

[144] Y. Xiao, H. Zhang, Z. Zhang, M. Yan, M. Lei, K. Zeng, C. Zhao, Synthesis of novel tetravalent galactosylated DTPA-DSPE and study on hepatocyte-targeting efficiency in vitro and in vivo, Int. J. Nanomedicine, 8 (2013) 3033-3050.

[145] S. Kawakami, C. Munakata, S. Fumoto, F. Yamashita, M. Hashida, Targeted delivery of prostaglandin E_1 to hepatocytes using galactosylated liposomes, J. Drug Target., 8 (2000) 137-142.

[146] C. Zhao, Q. Feng, Z. Dou, W. Yuan, C. Sui, X. Zhang, G. Xia, H. Sun, J. Ma, Local targeted therapy of liver metastasis from colon cancer by galactosylated liposome encapsulated with doxorubicin, PLoS One, 8 (2013) e73860.

[147] C. Díaz, E. Vargas, O. Gätjens-Boniche, Cytotoxic effect induced by retinoic acid loaded into galactosyl-sphingosine containing liposomes on human hepatoma cell lines, Int. J. Pharm., 325 (2006) 108-115.

[148] T. Terada, M. Iwai, S. Kawakami, F. Yamashita, M. Hashida, Novel PEG-matrix metalloproteinase-2 cleavable peptide-lipid containing galactosylated liposomes for hepatocellular carcinoma-selective targeting, J. Control. Release, 111 (2006) 333-342.

[149] M. Garg, B.R. Garg, S. Jain, P. Mishra, R.K. Sharma, A.K. Mishra, T. Dutta, N.K. Jain, Radiolabeling, pharmacoscintigraphic evaluation and antiretroviral efficacy of

stavudine loaded 99mTc labeled galactosylated liposomes, Eur. J. Pharm. Sci., 33 (2008) 271-281.

[150] A.F. Chambers, L.M. Matrisian, Changing views of the role of matrix metalloproteinases in metastasis, J. Natl. Cancer Inst., 89 (1997) 1260-1270.

[151] S. Curran, G.I. Murray, Matrix metalloproteinases in tumour invasion and metastasis, J. Pathol., 189 (1999) 300-308.

[152] Y. Murawaki, S. Yamada, Y. Ikuta, H. Kawasaki, Clinical usefulness of serum matrix metalloproteinase-2 concentration in patients with chronic viral liver disease, J. Hepatol., 30 (1999) 1090-1098.

[153] H. Hatakeyama, H. Akita, K. Kogure, M. Oishi, Y. Nagasaki, Y. Kihira, M. Ueno, H. Kobayashi, H. Kikuchi, H. Harashima, Development of a novel systemic gene delivery system for cancer therapy with a tumor-specific cleavable PEG-lipid, Gene Ther., 14 (2007) 68-77.

[154] Y. Sakurai, H. Hatakeyama, H. Akita, M. Oishi, Y. Nagasaki, S. Futaki, H. Harashima, Efficient short interference RNA delivery to tumor cells using a combination of octaarginine, GALA and tumor-specific, cleavable polyethylene glycol system, Biol. Pharm. Bull., 32 (2009) 928-932.

[155] H. Hatakeyama, H. Akita, H. Harashima, The polyethyleneglycol dilemma: advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors, Biol. Pharm. Bull., 36 (2013) 892-899.

[156] S. Kawakami, S. Fumoto, M. Nishikawa, F. Yamashita, M. Hashida, In vivo gene delivery to the liver using novel galactosylated cationic liposomes, Pharm. Res., 17 (2000) 306-313.

[157] S. Fumoto, F. Nakadori, S. Kawakami, M. Nishikawa, F. Yamashita, M. Hashida, Analysis of hepatic disposition of galactosylated cationic liposome/plasmid DNA complexes in perfused rat liver, Pharm. Res., 20 (2003) 1452-1459.

[158] E. Letrou-Bonneval, R. Chèvre, O. Lambert, P. Costet, C. André, C. Tellier, B. Pitard, Galactosylated multimodular lipoplexes for specific gene transfer into primary hepatocytes, J. Gene Med., 10 (2008) 1198-1209.

[159] S. Kawakami, Y. Ito, S. Fumoto, F. Yamashita, M. Hashida, Enhanced gene expression in lung by a stabilized lipoplex using sodium chloride for complex formation, J. Gene Med., 7 (2005) 1526-1533.

[160] S. Fumoto, S. Kawakami, Y. Ito, K. Shigeta, F. Yamashita, M. Hashida, Enhanced hepatocyte-selective in vivo gene expression by stabilized galactosylated liposome/plasmid DNA complex using sodium chloride for complex formation, Mol. Ther., 10 (2004) 719-729.

[161] S. Fumoto, S. Kawakami, K. Shigeta, Y. Higuchi, F. Yamashita, M. Hashida, Interaction with blood components plays a crucial role in asialoglycoprotein receptor-mediated in vivo gene transfer by galactosylated lipoplex, J. Pharmacol. Exp. Ther., 315 (2005) 484-493.

[162] E. Perouzel, M.R. Jorgensen, M. Keller, A.D.Miller, Synthesis and formulation of neoglycolipids for the functionalization of liposomes and lipoplexes, Bioconjug. Chem., 14 (2003) 884-898.

[163] J.S. Choi, J.A. MacKay, F.C. Szoka Jr., Low-pH-sensitive PEG-stabilized plasmid-lipid nanoparticles: preparation and characterization, Bioconjug. Chem., 14 (2003) 420-429.

[164] J.H. Felgner, R. Kumar, C.N. Sridhar, C.J. Wheeler, Y.J. Tsai, R. Border, P. Ramsey, M. Martin, P.L. Felgner, Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations, J. Biol. Chem., 269 (1994) 2550-2561.

[165] Y. Saito, S. Kawakami, Y. Yabe, F. Yamashita, M. Hashida, Intracellular trafficking is the important process that determines the optimal charge ratio on transfection by galactosylated lipoplex in HepG2 cells, Biol. Pharm. Bull., 29 (2006) 1986-1990.

[166] K. Shigeta, S. Kawakami, Y. Higuchi, T. Okuda, H. Yagi, F. Yamashita, M. Hashida, Novel histidine-conjugated galactosylated cationic liposomes for efficient hepatocyte-selective gene transfer in human hepatoma HepG2 cells, J. Control. Release, 118 (2007) 262-270.

[167] K.L. Chang, Y. Higuchi, S. Kawakami, F. Yamashita, M. Hashida, Development of lysine-histidine dendron modified chitosan for improving transfection efficiency in HEK293 cells, J. Control. Release, 156 (2011) 195-202.

[168] Y. Hu, M.T. Haynes, Y. Wang, F. Liu, L. Huang, A highly efficient synthetic vector: nonhydrodynamic delivery of DNA to hepatocyte nuclei in vivo, ACS Nano., 7 (2013) 5376-5384.

[169] S. Kawakami, M. Hashida, Targeted delivery systems of small interfering RNA by systemic administration, Drug Metab. Pharmacokinet., 22 (2007) 142-151.

[170] Y. Higuchi, S. Kawakami, M. Hashida, Strategies for in vivo delivery of siRNAs: recent progress, BioDrugs, 24 (2010) 195-205.

[171] A. Sato, M. Takagi, A. Shimamoto, S. Kawakami, M. Hashida, Small interfering RNA delivery to the liver by intravenous administration of galactosylated cationic liposomes in mice, Biomaterials, 28 (2007) 1434-1442.

[172] S. Sonoke, T. Ueda, K. Fujiwara, K. Kuwabara, J. Yano, Galactose-modified

cationic liposomes as a liver-targeting delivery system for small interfering RNA, Biol. Pharm. Bull., 34 (2011) 1338-1342.

[173] N. Jiang, X. Zhang, X. Zheng, D. Chen, Y. Zhang, L.K. Siu, H.B. Xin, R. Li, H. Zhao, N. Riordan, T.E. Ichim, D. Quan, A.M. Jevnikar, G. Chen, W. Min, Targeted gene silencing of TLR4 using liposomal nanoparticles for preventing liver ischemia reperfusion injury, Am. J. Transplant. , 11 (2011) 1835-1844.

[174] N. Jiang, X. Zhang, X. Zheng, D. Chen, K. Siu, H. Wang, T.E. Ichim, D. Quan, V. McAlister, G. Chen, W.P. Min, A novel in vivo siRNA delivery system specifically targeting liver cells for protection of ConA-induced fulminant hepatitis, PLoS One, 7 (2012) e44138.

[175] G. Barratt, J.P. Tenu, A. Yapo, J.F.Petit, Preparation and characterisation of liposomes containing mannosylated phospholipids capable of targetting drugs to macrophages, Biochim. Biophys. Acta, 862 (1986) 153-164.

[176] M. Moonis, I. Ahmad, BK. Bachhawat, Mannosylated liposomes as carriers for hamycin in the treatment of experimental aspergillosis in Balb/C mice, J. Drug Target., 1 (1993) 147-155.

[177] C.D. Muller, F. Schuber, Neo-mannosylated liposomes: synthesis and interaction with mouse Kupffer cells and resident peritoneal macrophages, Biochim. Biophys. Acta, 986 (1989) 97-105.

[178] M. Fukasawa, Y. Shimizu, K. Shikata, M. Nakata, R. Sakakibara, N. Yamamoto, M. Hatanaka, T. Mizuochi, Liposome oligomannose-coated with neoglycolipid, a new candidate for a safe adjuvant for induction of $CD8^+$ cytotoxic T lymphocytes, FEBS Lett., 441 (1998) 353-356.

[179] S. Kawakami, J. Wong, A. Sato, Y. Hattori, F. Yamashita, M. Hashida, Biodistribution characteristics of mannosylated, fucosylated, and galactosylated liposomes in mice, Biochim. Biophys. Acta, 1524 (2000) 258-265.

[180] A. Düffels, L.G. Green, S.V. Ley, A.D.Miller, Synthesis of high-mannose type neoglycolipids: active targeting of liposomes to macrophages in gene therapy, Chemistry, 6 (2000) 1416-1430.

[181] S. Espuelas, P. Haller, F. Schuber, B. Frisch, Synthesis of an amphiphilic tetraantennary mannosyl conjugate and incorporation into liposome carriers, Bioorg. Med. Chem. Lett., 13 (2003) 2557-2560.

[182] A. Engel, S.K. Chatterjee, A. Al-arifi, D. Riemann, J. Langner, P. Nuhn, Influence of spacer length on interaction of mannosylated liposomes with human phagocytic cells, Pharm. Res., 20 (2003) 51-57.

[183] K.L. White, T. Rades, R.H. Furneaux, P.C. Tyler, S. Hook, Mannosylated

liposomes as antigen delivery vehicles for targeting to dendritic cells, J. Pharm. Pharmacol., 58 (2006) 729-737.

[184] M. Garg, A. Asthana, H.B. Agashe, G.P. Agrawal, N.K. Jain, Stavudine-loaded mannosylated liposomes: in-vitro anti-HIV-I activity, tissue distribution and pharmacokinetics, J. Pharm. Pharmacol., 58 (2006) 605-616.

[185] S. Chono, T. Tanino, T. Seki, K. Morimoto, Uptake characteristics of liposomes by rat alveolar macrophages: influence of particle size and surface mannose modification, J. Pharm. Pharmacol., 59 (2007) 75-80.

[186] S. Espuelas, C. Thumann, B. Heurtault, F. Schuber, B. Frisch, Influence of ligand valency on the targeting of immature human dendritic cells by mannosylated liposomes, Bioconjug. Chem., 19 (2008) 2385-2393.

[187] S. Ghosh, N. Das, A.K. Mandal, S.R. Dungdung, S. Sarkar, Mannosylated liposomal cytidine 5' diphosphocholine prevent age related global moderate cerebral ischemia reperfusion induced mitochondrial cytochrome c release in aged rat brain, Neuroscience, 171 (2010) 1287-1299.

[188] F. Perche, T. Benvegnu, M. Berchel, L. Lebegue, C. Pichon, P.A. Jaffrès, P. Midoux, P. Enhancement of dendritic cells transfection in vivo and of vaccination against B16F10 melanoma with mannosylated histidylated lipopolyplexes loaded with tumor antigen messenger RNA, Nanomedicine, 7 (2011) 445-453.

[189] A. Rathore, A. Jain, A. Gulbake, S. Shilpi, P. Khare, A. Jain, SK. Jain, Mannosylated liposomes bearing Amphotericin B for effective management of visceral Leishmaniasis, J. Liposome Res., 21 (2011) 333-340.

[190] F. Kong, F. Zhou, L. Ge, X. Liu, Y. Wang, Mannosylated liposomes for targeted gene delivery, Int. J. Nanomedicine, 7 (2012) 1079-1089.

[191] L.W. Locke, M.W. Mayo, A.D. Yoo, M.B. Williams, S.S. Berr, PET imaging of tumor associated macrophages using mannose coated ^{64}Cu liposomes, PET imaging of tumor associated macrophages using mannose coated ^{64}Cu liposomes, Biomaterials, 33 (2012) 7785-7793.

[192] S. Kawakami, A. Sato, M. Nishikawa, F. Yamashita, M. Hashida, Mannose receptor-mediated gene transfer into macrophages using novel mannosylated cationic liposomes, Gene Ther., 7 (2000) 292-299.

[193] P. Opanasopit, M. Sakai, M. Nishikawa, S. Kawakami, F. Yamashita, M. Hashida, Inhibition of liver metastasis by targeting of immunomodulators using mannosylated liposome carriers, J. Control. Release, 80 (2002) 283-294.

[194] Y. Hattori, S. Suzuki, S. Kawakami, F. Yamashita, M. Hashida, The role of dioleoylphosphatidylethanolamine (DOPE) in targeted gene delivery with

mannosylated cationic liposomes via intravenous route, J. Control. Release, 108 (2005) 484-495.

[195] Y. Higuchi, S. Kawakami, M. Oka, Y. Yabe, F. Yamashita, M. Hashida, Intravenous administration of mannosylated cationic liposome/NFkappaB decoy complexes effectively prevent LPS-induced cytokine production in a murine liver failure model, FEBS Lett., 580 (2006) 3706-3714.

[196] Y. Kuramoto, S. Kawakami, S. Zhou, K. Fukuda, F. Yamashita, M. Hashida, Mannosylated cationic liposomes/CpG DNA complex for the treatment of hepatic metastasis after intravenous administration in mice, J. Pharm. Sci., 98 (2009) 1193-1197.

[197] H. Huang, F. Sakurai, Y. Higuchi, S. Kawakami, M. Hashida, K. Kawabata, H. Mizuguchi, Suppressive effects of sugar-modified cationic liposome/NF-κB decoy complexes on adenovirus vector-induced innate immune responses, J. Control. Release, 133 (2009) 139-145.

[198] Y. Hattori, S. Kawakami, K. Nakamura, F. Yamashita, M. Hashida, Efficient gene transfer into macrophages and dendritic cells by in vivo gene delivery with mannosylated lipoplex via the intraperitoneal route, J. Pharmacol. Exp. Ther., 318 (2006) 828-834.

[199] Y. Hattori, S. Kawakami, Y. Lu, K. Nakamura, F. Yamashita, M. Hashida, Enhanced DNA vaccine potency by mannosylated lipoplex after intraperitoneal administration, J. Gene Med., 8 (2006) 824-834.

[200] Y. Lu, S. Kawakami, F. Yamashita, M. Hashida, Development of an antigen-presenting cell-targeted DNA vaccine against melanoma by mannosylated liposomes, Biomaterials, 28 (2007) 3255-3262.

[201] Y. Kuramoto, S. Kawakami, S. Zhou, K. Fukuda, F. Yamashita, M. Hashida, Use of mannosylated cationic liposomes/ immunostimulatory CpG DNA complex for effective inhibition of peritoneal dissemination in mice, J. Gene Med., 10 (2008) 392-399.

[202] W. Wijagkanalan, Y. Higuchi, S. Kawakami, M. Teshima, H. Sasaki, M. Hashida, Enhanced anti-inflammation of inhaled dexamethasone palmitate using mannosylated liposomes in an endotoxin-induced lung inflammation model, Mol. Pharmacol., 74 (2008) 1183-1192.

[203] W. Wijagkanalan, S. Kawakami, Y. Higuchi, F. Yamashita, M. Hashida, Intratracheally instilled mannosylated cationic liposome/NFκB decoy complexes for effective prevention of LPS-induced lung inflammation, J. Control. Release, 149 (2011) 42-50.

[204] Y. Shimizu, H. Takagi, T. Nakayama, K. Yamakami, T. Tadakuma, N. Yokoyama, N. Kojima, Intraperitoneal immunization with oligomannose-coated liposome-entrapped soluble leishmanial antigen induces antigen-specific T-helper type immune response in BALB/c mice through uptake by peritoneal macrophages, Parasite Immunol., 29 (2007) 229-239.

[205] Y. Ikehara, N. Shiuchi, S. Kabata-Ikehara, H. Nakanishi, N. Yokoyama, H. Takagi, T. Nagata, Y. Koide, K. Kuzushima, T. Takahashi, K. Tsujimura, N. Kojima, Effective induction of anti-tumor immune responses with oligomannose-coated liposome targeting to intraperitoneal phagocytic cells, Cancer Lett., 260 (2008) 137-145.

[206] W. Yeeprae, S. Kawakami, F. Yamashita, M. Hashida, Effect of mannose density on mannose receptor-mediated cellular uptake of mannosylated O/W emulsions by macrophages, J. Control. Release, 114 (2006) 193-201.

[207] H.S. Jeong, C.M. Lee, S.J. Cheong, E.M. Kim, H. Hwang, K.S. Na, S.T. Lim, M.H. Sohn, H.J.Jeong, The effect of mannosylation of liposome-encapsulated indocyanine green on imaging of sentinel lymph node, J. Liposome Res., 23 (2013) 291-297.

[208] S. Kawakami, Y, Hattori, Y. Lu, Y. Higuchi, F. Yamashita, M. Hashida, Effect of cationic charge on receptor-mediated transfection using mannosylated cationic liposome/plasmid DNA complexes following the intravenous administration in mice, Pharmazie, 59 (2004) 405-408.

[209] P. Li, S. Chen, Y. Jiang, J. Jiang, Z. Zhang, X. Sun, Dendritic cell targeted liposomes-protamine-DNA complexes mediated by synthetic mannosylated cholesterol as a potential carrier for DNA vaccine, Nanotechnology, 24 (2013) 295101.

[210] K. Nakamura, Y. Kuramoto, H. Mukai, S. Kawakami, Y. Higuchi, M. Hashida, Enhanced gene transfection in macrophages by histidine-conjugated mannosylated cationic liposomes, Biol. Pharm. Bull., 32 (2009) 1628-1631.

[211] M. Mizuuchi, Y. Hirohashi, T. Torigoe, T. Kuroda, K. Yasuda, Y. Shimizu, T. Saito, N. Sato, Novel oligomannose liposome-DNA complex DNA vaccination efficiently evokes anti-HPV E6 and E7 CTL responses, Exp. Mol. Pathol., 92 (2012) 185-190.

[212] R. Suzuki, Y. Oda, N. Utoguchi, K. Maruyama, Progress in the development of ultrasound-mediated gene delivery systems utilizing nano- and microbubbles, J. Control. Release, 149 (2011) 36-41.

[213] R. Suzuki, T. Takizawa, Y. Negishi, K. Hagisawa, K. Tanaka, K. Sawamura, N. Utoguchi, T. Nishioka, K. Maruyama, Gene delivery by combination of novel

liposomal bubbles with perfluoropropane and ultrasound, J. Control. Release, 117 (2007) 130-136.

[214] K. Un, S. Kawakami, R. Suzuki, K. Maruyama, F. Yamashita, M. Hashida, Development of an ultrasound-responsive and mannose-modified gene carrier for DNA vaccine therapy, Biomaterials, 31 (2010) 7813-7826.

[215] K. Un, S. Kawakami, R. Suzuki, K. Maruyama, F. Yamashita, M. Hashida, Suppression of melanoma growth and metastasis by DNA vaccination using an ultrasound-responsive and mannose-modified gene carrier, Mol. Pharm., 8 (2011) 543-554.

[216] K. Un, S. Kawakami, M. Yoshida, Y. Higuchi, R. Suzuki, K. Maruyama, F. Yamashita, M. Hashida, The elucidation of gene transferring mechanism by ultrasound-responsive unmodified and mannose-modified lipoplexes, Biomaterials, 32 (2011) 4659-4669.

[217] K. Un, S. Kawakami, Y. Higuchi, R. Suzuki, K. Maruyama, F. Yamashita, M. Hashida, Involvement of activated transcriptional process in efficient gene transfection using unmodified and mannose-modified bubble lipoplexes with ultrasound exposure, J. Control. Release, 156 (2011) 355-363.

[218] K. Un, S. Kawakami, M. Yoshida, Y. Higuchi, R. Suzuki, K. Maruyama, F. Yamashita, M. Hashida, Efficient suppression of murine intracellular adhesion molecule-1 using ultrasound-responsive and mannose-modified lipoplexes inhibits acute hepatic inflammation, Hepatology, 56 (2012) 259-269.

[219] Y. Kono, S. Kawakami, Y. Higuchi, K. Maruyama, F. Yamashita, M. Hashida, Tumour-associated macrophage targeted NF-κB decoy transfection with mannose-modified bubble lipoplexes inhibits tumour growth in tumour bearing mice, J. Drug Targeting, 22 (2014) 439-449.

[220] T. Kurosaki, S. Kawakami, Y. Higuchi, R. Suzuki, K. Maruyama, H. Sasaki, F. Yamashita, M. Hashida, Development of anionic bubble lipopolyplexes for efficient and safe gene transfection with ultrasound exposure in mice, J Control Release, 176 (2014) 24-34.

[221] A.L. Klibanov, J.J. Rychak, W.C. Yang, S. Alikhani, B. Li, S. Acton, J.R. Lindner, K. Ley, S. Kaul, Targeted ultrasound contrast agent for molecular imaging of inflammation in high-shear flow, Contrast Media Mol. Imaging, 1 (2006) 259-266.

[222] E.A. Ferrante, J.E. Pickard, J. Rychak, A. Klibanov, K. Ley, Dual targeting improves microbubble contrast agent adhesion to VCAM-1 and P-selectin under flow, J. Control. Release, 140 (2009) 100-107.

[223] R.S. Haltiwanger, M.A. Lehrman, A.E. Eckhardt, R.L. Hill, The distribution and

localization of the fucose-binding lectin in rat tissues and the identification of a high affinity form of the mannose/N-acetylglucosamine-binding lectin in rat liver, J. Biol. Chem., 261 (1986) 7433-7439.

[224] Y. Higuchi, M. Nishikawa, S. Kawakami, F. Yamashita, M. Hashida, Uptake characteristics of mannosylated and fucosylated bovine serum albumin in primary cultured rat sinusoidal endothelial cells and Kupffer cells, Int. J. Pharm., 287 (2004) 147-154.

[225] Y. Higuchi, S. Kawakami, F. Yamashita, M. Hashida, The potential role of fucosylated cationic liposome/NFκB decoy complexes in the treatment of cytokine-related liver disease, Biomaterials, 28 (2007) 532-539.

[226] M. Yoshida, R. Takimoto, K. Murase, Y. Sato, M. Hirakawa, F. Tamura, T. Sato, S. Iyama, T. Osuga, K. Miyanishi, K. Takada, T. Hayashi, M. Kobune, J. Kato, Targeting anticancer drug delivery to pancreatic cancer cells using a fucose-bound nanoparticle approach, PLoS One, 7 (2012) e39545.

[227] C.H. Campbell, A.L. Miller, L.H. Rome, Incorporation of mannose 6-phosphate receptors into liposomes. Receptor topography and binding of α-mannosidase, Biochem. J., 214 (1983) 413-419.

[228] J.E. Adrian, K. Poelstra, G.L. Scherphof, G. Molema, D.K. Meijer, C. Reker-Smit, H.W. Morselt, J.A.Kamps, Interaction of targeted liposomes with primary cultured hepatic stellate cells: Involvement of multiple receptor systems, J. Hepatol., 44 (2006) 560-567.

[229] J.E. Adrian, K. Poelstra, G.L. Scherphof, D.K. Meijer, A.M. van Loenen-Weemaes, C. Reker-Smit, H.W. Morselt, P. Zwiers, J.A. Kamps, Effects of a new bioactive lipid-based drug carrier on cultured hepatic stellate cells and liver fibrosis in bile duct-ligated rats, J. Pharmacol. Exp. Ther., 321 (2007) 536-543.

[230] J.E. Adrian, J.A. Kamps, G.L. Scherphof, D.K. Meijer, A.M. van Loenen-Weemaes, C. Reker-Smit, P. Terpstra, K. Poelstra, A novel lipid-based drug carrier targeted to the non-parenchymal cells, including hepatic stellate cells, in the fibrotic livers of bile duct ligated rats, Biochim. Biophys. Acta, 1768 (2007) 1430-1439.

[231] J.E. Adrian, J.A. Kamps, K. Poelstra, G.L. Scherphof, D.K. Meijer, Y. Kaneda, Delivery of viral vectors to hepatic stellate cells in fibrotic livers using HVJ envelopes fused with targeted liposomes, J. Drug Target., 15 (2007) 75-82.

[232] D. Wakebayashi, N. Nishiyama, Y. Yamasaki, K. Itaka, N. Kanayama, A. Harada, Y. Nagasaki, K. Kataoka, Lactose-conjugated polyion complex micelles incorporating plasmid DNA as a targetable gene vector system: their preparation

and gene transfecting efficiency against cultured HepG2 cells, J. Control. Release, 95 (2004) 653-664.

[233] Y. Wang, C.Y. Hong, C.Y. Pan, Galactose-based amphiphilic block copolymers: synthesis, micellization, and bioapplication, Biomacromolecules, 14 (2013) 1444-1451.

[234] S.S. Yu, C.M. Lau, W.J. Barham, H.M. Onishko, C.E. Nelson, H. Li, C.A. Smith, F.E. Yull, C.L. Duvall, T.D. Giorgio, Macrophage-specific RNA interference targeting via "click", mannosylated polymeric micelles, Mol. Pharm., 10 (2013) 975-987.

[235] Y. Zou, Y. Song, W. Yang, F. Meng, H. Liu, Z. Zhong, Galactose-installed photo-crosslinked pH-sensitive degradable micelles for active targeting chemotherapy of hepatocellular carcinoma in mice, J. Control. Release, in press

[236] Y. Bae, K. Kataoka, Intelligent polymeric micelles from functional poly(ethylene glycol)-poly(amino acid) block copolymers, Adv. Drug Deliv. Rev., 61 (2009) 768-784.

[237] K. Itaka, K. Kataoka, Progress and prospects of polyplex nanomicelles for plasmid DNA delivery, Curr. Gene Ther., 11 (2011) 457-465.

[238]S. Hossain, A. Stanislaus, M.J. Chua, S. Tada, Y. Tagawa, E.H. Chowdhury, T. Akaike, Carbonate apatite-facilitated intracellularly delivered siRNA for efficient knockdown of functional genes, J. Control. Release, 147(2010) 101-108.

[239] S. Fumoto, S. Kawakami, Combination of nanoparticles with physical stimuli toward cancer therapy, Biol. Pharm. Bull, 37 (2014) 212-216.