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Targeted delivery of drugs for liver fibrosis

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Liver fibrosis and its end stage disease cirrhosis are a major cause of mortality and morbidity around the world. There is no effective pharmaceutical intervention for liver fibrosis at present. Many drugs that show potent anti-fibrotic activities *in vitro* often show only minor effects *in vivo* because of insufficient concentrations of drugs accumulating around the target cell and their adverse effects as a result of affecting other non-target cells. Hepatic stellate cells (HSC) play a critical role in the fibrogenesis of liver, so they are the target cells of antifibrotic therapy. Several kinds of targeted delivery system that could target the receptors expressed on HSC have been designed, and have shown an attractive targeted potential *in vivo*. After being carried by these delivery systems, many agents showed a powerful antifibrotic effect in animal models of liver fibrosis. These targeted delivery systems provide a new pathway for the therapy of liver fibrosis. The characteristics of theses targeted carriers are reviewed in this paper.

Keywords: antifibrosis, delivery systems, hepatic stellate cells, liver fibrosis, target

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1. Introduction

Liver fibrosis and its end stage disease cirrhosis are major world health problems arising from chronic injury of the liver by a variety of etiological factors. Worldwide, the common causes of liver fibrosis and cirrhosis include hepatitis B, hepatitis C and alcohol. Other causes include immune-mediated damage, genetic abnormalities and non-alcoholic steatohepatitis, which is associated with diabetes and the metabolic syndrome [1]. Changing patterns of alcohol consumption in the West and the increasing rates of obesity and diabetes mean that advances in preventing and treating viral hepatitis may be offset by an increasing burden of fibrosis and cirrhosis related to alcohol and non-alcoholic steatohepatitis [1,2]. Liver cirrhosis is characterized by extensive fibrous scarring of the liver and the formation of pseudo lobes, which are typically associated with such clinical signs as variceal hemorrhage, ascites, or hepatocellular carcinoma [3]. In the UK alone, the subsequent liver cirrhosis results in 4000 deaths a year, with two-thirds dying before their 65th birthday, and the incidence of cirrhosis-related death is increasing [4].

The key factor in the pathogenesis of liver fibrosis is the activation and proliferation of hepatic stellate cells (HSC) and their transformation into myofibroblasts. HSC contribute largely to the intrahepatic connective tissue expansion during fibrogenesis [5]. As a result of liver injury, HSC, which in the healthy organ store vitamin A, undergo a process of activation that is mediated by the concerted action of resident hepatic cell types such as Kupffer cells (KC), liver endothelial cells (LEC) and hepatocytes. The phenotype of activated HSC resembles that of myofibroblasts and is characterized by: α-smooth muscle actin (α-SMA) expression; intensive syntheses of extracellular matrix proteins, mainly type I and type III collagen; and a high rate

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of proliferation [6,7]. Activated HSC also secrete pro-fibrotic and pro-inflammatory mediators, which, in an autocrine manner, perpetuate the activated state of HSC and attract immune cells from the bloodstream [8,9]. In addition, the contractile features of activated HSC are the basis for their pivotal role in the portal hypertension, which is a major clinical characteristic of liver cirrhosis [10].

Current treatments for cirrhosis are limited to removing the underlying injurious stimulus (where possible), eradicating viruses using interferon, ribavirin and nucleoside analogues in viral hepatitis, and liver transplantation. Transplantation is a highly successful treatment for end stage cirrhosis, with a 75% 5-year survival rate [2]; but limited availability of organs, growing lists of patients needing a transplant, issues of compatibility and comorbid factors mean that not everyone is eligible for transplantation. As a result, effective antifibrotic treatments are needed urgently. As HSC have a crucial role in hepatic fibrogenesis, they are attractive targets for antifibrotic therapy. Pharmacotherapeutic intervention of HSC functions can take place at different levels: inhibition of the activation and transformation of HSC, inactivation of profibrogenic cytokines, interference with matrix synthesis and stimulation of matrix degradation [5].

Despite potent activities of many antifibrotic drugs *in vitro*, often only minor effects are observed *in vivo*. So far, no pharmaceutical intervention is available to treat this fibrotic disease [11]. The main problem with these drugs *in vivo* is that they are neither liver-specific nor fibrosis-specific. As a consequence, insufficient concentrations of drugs could be accumulated in the target cells. Moreover, uptake of the drug in non-target cells could in principle cause serious adverse effects, in particular during chronic administration [12]. Targeting therapeutics to the relevant tissue or disease-associated cell has several advantages over non-targeted therapies. Lower levels of therapeutic need to be delivered to achieve therapeutic effects, and altered pharmacodynamics increase the likelihood of a specific action in the target tissue/cell and reduce the chances of adverse effects [13-15]. The principal cell type responsible for hepatic fibrogenesis is the activated HSC. Therefore, this cell type is an important target for antifibrotic therapies. To attain HSC-specific uptake, drugs can be coupled to carrier molecules that are designed for selective uptake by the target cells. Receptors expressed on (activated) HSC may serve as targets for such carriers [16]. A variety of small molecules, such as peptides, carbohydrates or antibody fragments, can be selected for specific target ligands with 'one-bead one-compound' combinatorial libraries [17].

In the past decade, several kinds of delivery system targeting to activated HSC or other non-parenchymal cells involved in liver fibrogenesis have been designed and explored. Most of them have shown an attractive potential in antifibrotic therapy, and agents carried by them showed a powerful antifibrotic effect *in vivo*. These delivery systems are reviewed in this article.

2. Human serum albumin modified with mannose 6-phosphate

Binding sites expressed on (activated) HSC are considered for their ability to serve as potential targets for carrier molecules. One of these is the mannose 6-phosphate/insulinlike growth factor II (M6P/IGF-II) receptor, whose expression is increased on activated rat HSC, particularly during fibrosis [18,19]. The receptor has binding sites for IGF-II and M6P-containing ligands such as latent transforming growth factor-β (L-TGFβ), proliferin and lysosomal enzymes [20].

Beljaars *et al.* took the lead to demonstrate that human serum albumin (HSA) modified with M6P could be taken up by HSC in fibrotic livers. A series of $M6P_x$-modified ($x = 2$, 4, 10 and 28) albumins were synthetized. The hepatic uptake of $M6P_x$-HSA increased with increasing M6P density. $M6P_x$-HSA with a low degree of sugar loading ($x = 2 - 10$) remained in the plasma and accumulated less in fibrotic rat livers. When the molar ratio of M6P:HSA was increased to 28:1, an increased liver accumulation to 59 ± 9% of the administered dose was observed; and, *in vivo*, an increased substitution of M6P was associated with an increased accumulation in HSC; 70 ± 11% of the intrahepatic staining for $M6P_{28}$-HSA was found in HSC. Furthermore, the M6P-modified serum albumin also accumulated in slices of normal and cirrhotic human livers. After incubation of this neoglycoprotein with human tissue, the protein was found in non-parenchymal liver cells. In normal human livers, LEC were responsible for the uptake of the protein, whereas in cirrhotic human livers, HSC, in particular to the activated phenotype, and LEC contributed to the uptake of this neoglycoprotein [5,21]. This binding of $M6P_{28}$-HSA and HSC was specific, as M6P and an excess of M6P-HSA inhibited the binding, which indicates the involvement of the M6P/IGF-II receptor, because M6P is a known ligand for this receptor. So mannose 6-phosphate groups attached to HSA (M6P-HSA) exert specificity for the M6P/IGF-II receptors present on HSC.

Approximately 80% of $M6P_{28}$-HSA synthetized is in the monomeric form and the coupling of M6P groups to albumin results in a more negatively charged protein as compared with unmodified HSA. The retention time is increased from 21.5 min for HSA to 27.2 min for $M6P_{28}$-HSA [21].

The negative charge of M6P-HSA, caused by the clustering of M6P groups on the protein core, could also qualify M6P-HSA as a ligand for scavenger receptors (ScR). Little is known about the expression of ScR on HSC. Schneiderhan *et al.* reported the expression of ScR class B on activated human HSC and demonstrated that oxidatively modified lipoproteins stimulate extracellular matrix synthesis through this receptor in HSC [22]. Adrian *et al.* explored the interaction of M6P-HSA liposomes with cultured HSC, and it was demonstrated that coupling M6P-HSA to the surface of liposomes significantly increased binding and intracellular uptake of these liposomes by cultured primary HSC. Surprisingly, a higher association of the M6P-HSA liposomes was observed for quiescent HSC

than for activated HSC, yet the results of RT-PCR and immunohistochemical staining showed an abundance of the M6P/IGF-II receptor on activated only [23]. Liposomes coupled with aconitylated HSA (AcoHSA) have been shown to associate readily with LEC through the ScR [24]. Adrian *et al.* found that the association of AcoHSA liposome with HSC was comparable to that of M6P-HSA liposomes. The associations of M6P-HSA liposomes and AcoHSA liposomes with LEC were both in the same order of magnitude. The expression of the M6P/IGF-II receptor by LEC was only at the mRNA, but not at the protein level. All of these results indicate that besides the M6P/IGF-II receptors, the ScR also play a role in the association of M6P-HSA with LEC and HSC [23]. Furthermore, the scavenger receptor competitor polyinosinic acid abolished the uptake of M6P-HSA liposomes and AcoHSA liposomes by LEC, which indicates that not ScR class B, but ScR class A, contributes to the association of these liposomes [25]. Although ScR class A is mainly expressed by macrophages, it is also present on endothelial cells, smooth muscle cells and fibroblasts [26,27].

Anti-fibrotic drugs display their pharmacological activities either inside the cell or at the cell membrane [28]. Therefore, it is important to determine whether M6P-HSA is endocytosed into HSC or remains at the cell membrane. The natural ligands for the M6P/IGF-II receptor undergo endocytosis after binding to the receptor [29]. M6P-HSA was demonstrated to be rapidly and extensively taken up into HSC through a lysosomal route. Endocytosis of M6P-HSA liposomes by HSC was temperature dependent and could be inhibited by monensin, a kind of inhibitor for endocytosis. Despite extensive internalization in lysosomes of activated HSC (up to 40% of the dose administered) only minor degradation products were detected. This indicates minor cellular processing of the modified albumin or retention of degradation products within the hepatic cells [21].

According to these results, M6P-HSA have been explored for the targeted delivery effect *in vivo*, either as a targeted carrier that conjugates directly with antifibrotic drugs, or as part of a targeted carrier, such as the M6P-HSA liposomes mentioned, which encapsulates antifibrotic drugs or genes inside. After conjugating directly with M6P-HSA, 18β-glycyrrhetinic acid, gliotoxin, pentoxifylline, losartan and the antiproliferative drug doxorubicin showed a significantly antifibrotic effect in animal models of liver fibrosis [30-34]. By doing so, accumulation of doxorubicin in extrahepatic tissues as well as hepatocytes was reported to be avoided [34].

Viral gene delivery systems might be an alternative to conventional treatment. The hemagglutinating virus of Japan (HVJ), also known as Sendai virus, is able to fuse with conventional liposomes, even though they do not contain sialoglycoproteins on their surface. During this fusion process, membrane proteins of HVJ are incorporated into the liposomal membrane. Several reports have shown that vectors derived from the fusion of liposomes that contain plasmid DNA or oligonucleotides with inactivated HVJ form an effective gene transfection system both *in vitro* and *in vivo* [35,36]. Liposomes that were surface coupled with M6P-HSA have been demonstrated to accumulate in HSC in a rat model of liver fibrosis [23]. So the fusion of HVJ-E with M6P-HSA liposomes creates new targeted particles for gene treatment, which in an experimental model of liver fibrosis have been shown to accumulate in HSC [37]. The combination of HSC specific targeting with the effective HVJ gene transfection system may offer the possibility to construct a new vector, characterized by the delivery of therapeutic genes selectively to HSC and an enhanced transfection efficiency of these pivotal cells during fibrogenesis.

As mentioned above, however, besides the M6P/IGF-II receptors, ScR can also bind with M6P-HSA. As ScR is expressed on HSC, LEC and KC, the antifibrotic drugs or genes carried by M6P-HSA may also have an effect on LEC and KC. After incorporating the bioactive lipid dilinoleoylphosphatidylcholine (DLPC) into the membrane of liposomes and coupling M6P-HSA to the surface of these liposomes, the effect of M6P-HSA-DLPC liposomes was evaluated on HSC activation and liver fibrosis. Targeted M6PHSA-DLPC liposomes and DLPC liposomes significantly reduced gene expression levels for collagen Iα1, α-SMA and TGF-β in cultured HSC. In fibrotic livers, DLPC liposomes decreased gene expression for TGF-β and collagen Iα1 as well as α-SMA and collagen protein expression. By contrast, M6PHSA-DLPC liposomes enhanced expression of pro-fibrotic and pro-inflammatory genes *in vivo*. In cultured Kupffer and endothelial cells, M6P-HSA liposomes influenced the expression of pro-inflammatory genes. So in fibrotic livers, M6P-HSA-mediated activation of Kupffer and endothelial cells probably counteracts this beneficial effect of DLPC liposomes [38].

3. Cyclic Arg-Gly-Asp peptides

Specific interactions between cells and extracellular matrix components are mediated by transmembrane proteins, in particular the heterodimeric integrins [39,40]. Proteins that contain the Arg-Gly-Asp (RGD) attachment site, together with the integrins that serve as receptors for them, constitute a major recognition system for cell adhesion. The RGD sequence is the cell attachment site of a large number of adhesive extracellular matrix, blood and cell surface proteins, and nearly half of the > 20 known integrins recognize this sequence in their adhesion protein ligands. Type VI collagen is a major matrix protein involved in the adhesion of cells to the surrounding matrix. The main cellular sources of type VI collagen in normal and fibrotic livers are HSC [41]. In normal livers, type VI collagen is localized in the portal areas and at the plasma membranes of hepatocytes, endothelial cells and HSC within the lobule. The hepatic deposition of this type of collagen is increased during liver fibrosis, in particular in the fibrous septa that invade the lobule [42]. RGD sequences are found in type VI collagen. The integrin-binding activity of adhesion proteins can be reproduced by short synthetic peptides containing the RGD

sequence. Reagents that bind selectively to only one or a few of the RGD-directed integrins can be designed by cyclizing peptides with selected sequences around the RGD and by synthesizing RGD mimics [39]. Although several RGD sequences are found in type VI collagen, the cyclic peptide C*GRGDSPC* has been shown to inhibit specifically the attachment of type VI collagen to cells, whereas the RGD-dependent binding of fibronectin to these cells was not inhibited and the linear analogue of this peptide failed to inhibit the cellular attachment of type VI collagen [43].

It has been confirmed that cyclic RGD peptide can target to rat HSC by recognizing the type VI collagen receptor [16,44]. Beljaars *et al.* modified HSA with 10 cyclic peptide moieties recognizing type VI collagen receptors (C*GRGDSPC*, in which C* denotes the cyclizing cysteine residues) yielding pCVI-HSA. *In vivo* experiments showed preferential distribution of pCVI-HSA to both fibrotic and normal rat livers. pCVI-HSA predominantly bound to HSC in fibrotic livers. By contrast, LEC contributed mostly to the total liver accumulation in normal rats. *In vitro* studies showed that pCVI-HSA bound specifically to rat HSC, in particular to the activated cells, and the amount of pCVI-HSA incorporated in HSC declined to ~ 50% with monensin, indicating internalization of pCVI-HSA by means of an endosomal and lysosomal pathway [16,44]. The results demonstrate the specificity of the stellate cell targeting and imply applicability of pCVI-HSA as carriers for drugs that act intracellularly.

Expect HSA, with sterically stable liposomes (SSL) that have been modified with cyclic RGD peptides (C*GRGDSPK*, in which C* and K* denote the cyclizing cysteine residues) to yield a new carrier, RGD-SSL. Interferon-α_{1b} (IFN-α_{1b}) or hepatocyte growth factor (HGF) could be trapped in SSL. When incubating HSC or hepatocytes with cyclic RGD peptide, the peptide was bound preferentially to activated HSC. A biodistribution study showed that the accumulation of RGD-SSL in HSC isolated from liver fibrotic rats was 10-fold more than with unlabeled SSL. The liver fibrotic rats receiving injections of IFN-α_{1b} or HGF trapped in RGD-SSL showed significantly reduced extent of liver fibrosis compared with liver fibrotic rats treated with IFN-α_{1b} or HGF trapped in SSL [45,46]. Different from the cyclic RGD peptides synthesized by Beljaars [16], the RGD peptides used to modify SSL modified the sequence by replacing cysteine with lysine, and the modified peptide was easily conjugated to the liposomal formulation by means of a sulf hydryl group in the cysteine residue. Furthermore, the modified cyclic RGD peptide, which formed a cycle with a peptide bond (–NH–CO–) between the lysine and cysteine residues, tended to form a more stable cyclic peptide than the original one, which was cyclized by forming an unstable disulfo bond (–S–S–) between two neighboring cysteine residues, because the peptide bond is much more stable than the disulfo bond owing to a lower possibility of being oxygenized [45].

4. Recombinant human monoclonal antibody fragment to synaptophysin

Synaptophysin (SYN) is a major transmembrane glycoprotein of small (30 – 80 nm) electron-translucent (SET) vesicles. This class of vesicles includes the presynaptic vesicles in neuronal cells and the somewhat larger synaptic-like microvesicles in neuroendocrine cells [47]. The SYN protein is known to be present in membranes of neuronal synaptic vesicles containing neurotransmitters, SYN-positive secretory granules in pancreatic tissue demonstrated to contain glucagons or insulin. Tumors of bronchial epithelium and gastrointestinal mucosa can also demonstrate SYN reactivity [48-51]. In the liver, SYN expression was reported to be restricted to HSC-derived myofibroblasts (myofibroblasts express several genes associated with neural tissue) [52,53]. In 1999, Cassiman *et al.* demonstrated that SYN was a new marker for quiescent as well as activated HSC. They found that SYN reactivity was present in perisinusoidal stellate cells in both human and rat normal liver biopsies. The number of SYN-reactive perisinusoidal cells increased in pathological conditions. Double staining for α-SMA and SYN demonstrated unequivocally colocalization of both markers in lobular stellate cells. In addition, freshly isolated rat stellate cells expressed SYN mRNA and protein; and the presence of small electron translucent vesicles in stellate cell projections was comparable to the SYN-reactive synaptic vesicles in neurons [53].

SYN's external cellular location and cycling to intracellular location(s) make it a potential site for targeting liver myo fibroblasts with therapeutics. Recombinant human monoclonal single chain antibodies (scAbs) were therefore generated to a conserved peptide sequence present in an extracellular domain of SYN [54,55]. Phage display was used to generate a human monoclonal antibody fragment to a peptide sequence present on an extracellular domain of SYN. An antibody fragment was isolated (termed C1-3), expressed in bacteria and purified. Fluorescently labeled C1-3 associates with human HSC but not hepatocytes in culture. Binding of fluorescently labeled C1-3 to HSC was blocked by the extracellular SYN peptide sequence, and uptake of the antibody intracellularly was inhibited by monensin. The toxin tributyltin – when conjugated to C1-3 – retained the ability to kill HSC, confirming that C1-3 is sequestered intracellularly [54]. In addition, Douglass *et al.* demonstrated that the C1-3 scAb did not cross the blood brain barrier and targeted liver myofibroblasts *in vivo*. C1-3 specifically targeted to α-SMA-positive liver myofibroblasts within scar regions of the liver *in vivo* and did not colocalize with liver monocytes/macrophages. C1-3-targeted gliotoxin, an NF-κB inhibitor, stimulated a fivefold increase in non-parenchymal cell apoptosis, depleted liver myofibroblasts by 60%, did not affect the number of monocytes/macrophages and significantly reduced fibrosis severity [55].

These results demonstrate the targeted effect of scAbs to SYN in the therapy for liver fibrosis. The antifibrotic effect of drugs that act intracellularly and are not effective enough

in vivo or display serious extrahepatic side effects is enhanced after conjugation with the scAbs.

5. HSA modified with platelet-derived growth factor receptor-recognizing peptide

A prominent stimulator of fibroblast proliferation during fibrosis is platelet-derived growth factor (PDGF) [56]. PDGF is a dimeric molecule composed of A and/or B chains. The PDGF isoforms (-AA, -AB, or -BB) exert their effects on target cells by binding to two structurally different receptors. The PDGF-α receptor binds to both A and B chains with high affinity, but the PDGF-β receptor binds only to the B chain [9,57,58]. In liver fibrosis, PDGF is the most important mediator involved in the proliferation and activation of HSC, which transform subsequently into myofibroblasts. The local production of PDGF is increased during fibrosis, and the *de novo* expression of the PDGF-β receptor is strongly enhanced on activated HSC [59-62].

The general event of the induction of PDGF-β receptors on cell membranes of fibroblasts after the onset of fibrosis prompted exploration of the possibility of developing a drug carrier that distributes to this receptor in order to obtain homing to the target cells in fibrotic tissues. To create a drug carrier that distributes specifically to fibrogenic cells, a PDGF receptor-recognizing peptide is coupled to HSA (pPB-HSA). This new peptide (C*SRNLIDC*, in which C* denotes the cyclizing cysteine residues) contains the amino acids arginine (R) and isoleucine (I) as the receptor-binding moieties of the PDGF B chain [63], together with the amino acids flanking these moieties in the native growth factor.

In rats with liver fibrosis, pPB-HSA quickly accumulated in the liver in contrast to unmodified HSA. The major part of pPB-HSA in the fibrotic liver was localized in HSC. The binding of pPB-HSA to HSC *in vivo* was confirmed *in vitro* with primary cultures of quiescent and activated HSC. After 2 h of incubation, only the activated HSC significantly bound pPB-HSA as compared with HSA. The cell-bound pPB-HSA at 37°C was not significantly higher than at 4°C, which indicated that no internalization occurred. So pPB-HSA may be used to deliver drugs that act extracellularly, such as receptor antagonists, and at the same time can deliver therapeutic agents to the desired site of action (dual targeting). In addition, pPB-HSA was able to reduce PDGF-BB-induced fibroblast proliferation *in vitro*, and proved to be devoid of proliferation-inducing activity itself [44,64]. Furthermore, the specificity of the binding of labeled pPB-HSA to activated HSC was examined, and it was shown that the amount of cell-bound radioactivity was decreased by 80% in the presence of an excess of unlabeled pPB-HSA. In the presence of the PDGF receptor antagonists suramin and trapidil, the binding of pPB-HSA to HSC declined to, respectively, 20 and 42% of the total binding. Moreover, the binding of pPB-HSA to cells was significantly lowered in the presence of PDGF-BB, but not by PDGF-AA, showing the specificity of the binding of pPB-HSA to PDGF-β receptors on activated HSC [64].

The cyclic peptides attached to HSA displayed a better interaction with the receptor than the cyclic peptide alone because the uncoupled peptides were not able to compete with the cellular binding of PDGF-BB. Apparently, exposition of more than one cyclic peptide on the albumin molecule more closely mimics the binding sites present in the PDGF molecule, which is a dimeric molecule [64,65]. Covalent attachment of several cyclic peptides to a backbone, such as HSA, may provide a better presentation to the receptor than the solubilized monomeric peptides. Another advantage of coupling the peptides to a macromolecule such as HSA is the prevention of rapid renal excretion that usually occurs with low molecular mass compounds.

15d-Prostaglandin J2 (15dPGJ2) induced apoptosis of human hepatic myofibroblasts and significantly inhibited the expression of interstitial collagens as well as their proliferation in human hepatic myofibroblasts *in vitro* [66-68]; but prostaglandins are locally acting mediators that are rapidly metabolized, resulting in a very short half-life [69]. In addition, the high protein binding of prostaglandins in serum might prevent 15dPGJ2 from effectively reaching the essential cells within the liver after systemic administration. *In vitro* studies demonstrated that pharmacological effects of 15dPGJ2 were abolished in the presence of serum [66]. The coupling of 15dPGJ2 to pPB-HSA clearly led to a rapid and near complete accumulation of the prostaglandin in the fibrotic liver with significant uptake in HSC. The targeted forms of 15dPGJ2 were pharmacologically effective *in vitro*, and coupling evidently improved the activity of this prostaglandin in the presence of serum [70].

The interaction of the macromolecule pPB-HSA with the PDGF receptor makes this modified albumin in principle applicable as a drug carrier to any fibrotic organ, in particular to the fibroblast-like cells in these organs. The homing of pPB-HSA was tested in rat models of kidney fibrosis as well [64]. It may also be applicable in other proliferative and inflammatory diseases associated with an upregulation of the PDGF-β receptor, such as pancreatic fibrosis, atherosclerosis and inflammatory joint disease. In these diseases, the fibroblasts are the main pathogenic cells, responsible for the production of the excessive amounts and deposition of aberrant forms of extracellular matrix, and therefore these cells are the main target cells for antifibrotic drugs.

6. Retinol-binding protein–retinol complex

Retinol is stored in liver. Hepatocytes initially take up the newly absorbed vitamin A from the diet, which is transported by blood circulation in chylomicron remnants. They subsequently transfer retinol to HSC, which store vitamin A in large cytoplasmic lipid droplets. HSC are thus specific retinoid-storing liver cells, and they take a leading part in the retinoid metabolism and homeostasis. The dynamic balance between

the accumulation and mobilization of systemic liver retinol deposits is primarily regulated in HSC. Circulating retinol is associated with plasma retinol-binding protein (RBP) or bovine serum albumin (BSA). HSC, but not myofibroblasts, expressed a high-affinity membrane receptor for RBP–retinol complex, and both cell types expressed a low-affinity one [71,72].

Sato *et al*. designed vitamin A-coupled liposomes to deliver small interfering RNA (siRNA) against gp46 (VA-lip-siRNAgp46), the rat homolog of human heat shock protein 47 (HSP47), to HSC [73]. The HSP47, collagen-specific chaperone facilitates collagen secretion by ensuring proper triple-helix formation of procollagen in the endoplasmic reticulum and has also been implicated in translational regulation of procollagen synthesis [74,75]. After culturing for 30 min, rat HSC treated with VA-lip-siRNAgp46 labeled with carboxyfluorescein (FAM) showed increasing fluorescence intensities as RBP concentrations increased, and the intensity plateaued at concentrations > 0.7 mg/ml RBP. To confirm specific uptake of vitamin A-coupled liposome/RBP complex by RBP receptors, anti-RBP antibody was added to the incubation medium of HSC treated with VA-lip-siRNAgp46-FAM in the presence of RBP (0.7 mg/ml). The uptake of siRNAgp46 by these cells was suppressed by anti-RBP antibody to levels near those with lip-siRNAgp46-FAM. In rats with liver fibrosis, VA-lip-siRNAgp46 quickly accumulated in the liver and the major part of VA-lip-siRNAgp46 in the fibrotic liver was localized in HSC. Both the HSC and the fibrotic livers of rats injected with the VA-lip-siRNAgp46 showed suppressed gp46 expression for at least 3 days. Five treatments with VA-lip-siRNAgp46 almost completely resolved liver fibrosis and prolonged survival in rats with lethal dimethylnitrosamine-induced liver cirrhosis in a dose- and duration-dependent manner. Rescue was not related to off-target effects or associated with recruitment of innate immunity. The receptor-specific siRNA delivery was similarly effective in suppressing collagen secretion and treating fibrosis induced by CCl4 or bile duct ligation [73]. These results demonstrate the effect of VA-lip as a targeted delivery system in liver fibrosis, and the effect depended on the existence of RBP.

As mentioned above, dietary retinoids delivered to the liver are first processed by hepatocytes, the major parenchymal cell type, and then transferred to HSC by means of a retinol-binding protein-dependent pathway [71]. Thus, it is interesting that the vitamin A-coupled liposomes were not first recognized by hepatocytes but instead delivered intact directly to HSC bound to retinol-binding protein. The mechanism needs to be explored further.

7. Recombinant adeno-associated virus (serotype 2)

Gene therapy represents an attractive approach for the treatment of liver fibrosis [76,77]. However, a cell-specific gene delivery system and a promising biological basis for the control of liver fibrogenesis are the main considerations for its clinical application. Among the currently available viral vectors, adeno-associated virus has several advantages, including stable transgene expression, which is particularly important for the treatment of chronic diseases [78].

The binding activities of recombinant adeno-associated virus (rAAV) (serotype 2) depend on the expression of its receptor and co-receptors on the cell surface. So far, one receptor and two co-receptors of rAAV have been identified [79-81]. The expression pattern of rAAV's receptor, heparan sulfate, remained unchanged in both normal and fibrotic livers. Interestingly, a dramatically increased expression of its co-receptor, fibroblast growth factor receptor-1α (FGFR-1α), was detected predominantly in the fibrotic areas of CCl4-induced livers with cirrhosis compared with normal livers, whereas no expression of FGFR-1α was detected in hepatocytes. By contrast, the expression pattern of integrin $\alpha_V\beta_5$ (another rAAV co-receptor) remained largely unchanged in a comparison of normal livers and livers with fibrosis. The expression of integrin $\alpha_V\beta_5$ was mainly restricted to hepatocyte and biliary epithelial cells. Impressively, detection of the localization of rAAV 2 h after portal injection showed a cluster of injected rAAV in the fibrotic areas of livers that was consistent with the expression pattern of FGFR-1α in the same setting. The association of FGFR-1α expression and the binding of injected rAAV were determined further by a double immunofluorescent staining in which the expression of FGFR-1α was colocalized with the presence of rAAV in the same cell. This suggests a preferential binding of rAAV to the cells in fibrotics areas of livers, which is associated with higher expression levels of its co-receptor FGFR-1α. Isolation of HSC from livers with fibrosis showed a higher expression of FGFR-1α at the protein of cellular membrane fraction. This suggests a correlation between higher levels of FGFR-1α expression and the preferential binding of rAAV to HSC in livers with fibrosis. This was supported further by the data associated with the patterns of transgene expression in isolated HSCs from livers with fibrosis. Heme oxygenase-1 (HO-1), which was primarily described as an antioxidant gene, was transduced with rAAV in rats of liver fibrosis. Isolation of different types of cells from CCl4-induced fibrotic livers showed predominant expression of transgene in HSC after rAAV/HO-1 administration on day 3 and remained stable for 12 weeks. In addition, HO-1-transduced HSC showed reduced transcript levels of type 1 collagen and impaired proliferative ability compared with controls. With this approach, the severity of established micronodular cirrhosis was markedly reduced [82].

The study demonstrates a natural tropism of rAAV vector with high efficiency in the transduction of target gene to HSC. A higher binding activity and transduction efficiency of injected rAAV to HSC could be based on the expression patterns of the rAAV receptor and co-receptors in livers with fibrosis. This observation, together with fewer immunogenic and desirable safety features, makes rAAV an ideal viral vector system for liver fibrosis gene therapy.

8. The targeted delivery system to LEC and KC

Kupffer and endothelial cells play an important role in the induction and progression of liver fibrosis through the release of a cascade of inflammatory mediators [83,84]. Consequently, these cells are the main targets for anti-inflammatory drugs, such as glucocorticosteroids, leukotriene inhibitors or radical scavenging agents.

The endothelial and Kupffer cells contain ScR-recognizing macromolecules with negative charge [85-88]. The affinity for ScR is also determined by the hydrophobicity of the protein conjugate [89]. Kamps *et al.* modified HSA with *cis*-aconitic anhydride, which covalently coupled to liposomes with a size of ~ 100 nm (polyaconitylated HSA [Aco-HAS] liposomes). Within 30 min of injection into a rat, Aco-HSA liposomes were completely cleared from the blood and taken up almost exclusively by the liver. LEC were shown to account for almost two-thirds of the hepatic uptake of the Aco-HSA liposomes, the remainder being recovered mainly in KC. Uptake of Aco-HSA liposomes by both endothelial and Kupffer cells was inhibited by preinjection with polyinosinic acid, indicating the involvement of ScR in the uptake process. The uptake of Aco-HAS liposomes by LEC was dependent on liposome size; with increasing liposome diameter endothelial cell uptake decreased in favor of KC uptake [90]. Aco-HSA can be particularly taken up by LEC and KC, introducing extra negative charges by reacting albumin with succinic anhydride (suc-HSA), leading to specific uptake of these polyanionic carrier molecules by means of ScR on endothelial cells as well [91,92]. Mannose receptors present on Kupffer cells are involved in the receptor-mediated endocytosis of mannosylated HSA (man-HSA), a process that is determined by the sugar density, net charge and added hydrophobicity through coupling of drugs to the mannosylated albumin [92,93]. Moreover, in fibrotic livers, selective cellular uptake of these drug carriers in LEC and KC was not altered in spite of the abundant collagen deposition in these livers [94].

The cell-selective hepatic delivery of drugs can be achieved by covalent linking of drugs to HSA. The coupling of Dexamethasone (Dexa) to HSA led to a shift in its distribution pattern from hepatocyte uptake to uptake by Kupffer and endothelial cells [95]. The coupling of Dexa to lysine groups in HSA prevents subsequent protonation of the lysine amino group, leading to an increase in net negative charge. In addition, the linking of Dexa molecules also increased the hydrophobicity of HSA. A preliminary study into the mechanism of the uptake of Dexai*-HSA by the non-parenchymal cells showed that poly-inosinic acid, a well-known antagonist of ScR, inhibited uptake of the conjugate, indicating that ScR were likely to be involved. The Dexai*-HSA conjugate was taken up intracellularly and released as active Dexa, as demonstrated in an *in vitro* system with liver slices. This was confirmed by the protective effect of Dexa-HSA in fibrotic rats challenged with LPS *in vivo.* The conjugate was indeed more effective at inhibiting LPS-induced TNF-α formation than unconjugated Dexa: 38% inhibition versus no inhibition of the TNF-α response, respectively, at the lowest concentration of the corticosteroid. In addition, a dose of 0.1 mg/kg Dexa-HSA 3 times a week reduced intrahepatic reactive oxygen species production strongly as compared with untreated bile duct ligation (BDL) rats. This dose, however, also stimulated the depositions of type I and type III collagens. Overdosing of $Dexa_{10}$-HSA (10 mg/kg) led to a lethal reduction of body and spleen weight. So Dexa-HSA has potent anti-inflammatory effects during BDL at extremely low doses, demonstrating cell-specific targeting. However, the fibrotic process was not favorably affected [96].

The above data imply the applicability of sugar- and charge-modified albumins as drug carriers for targeted delivery of antifibrotic drugs to LEC and KC involved in the fibrotic process. However, KC play different roles in different periods of liver fibrosis [97]. In 2005, Duffield *et al.* investigated macrophage function mechanistically in a reversible model of liver injury in which the injury and recovery phases were distinct. Macrophage depletion when liver fibrosis was advanced resulted in reduced scarring and fewer myofibroblasts. Macrophage depletion during recovery, by contrast, led to a failure of matrix degradation. These data provide the first clear evidence that functionally distinct subpopulations of macrophages exist in the same tissue and that these macrophages play critical roles in both the injury and recovery phases of inflammatory scarring [97]. These data also provide an explanation for Dexa-HAS mentioned above, which showed anti-inflammatory effects but not with an anti fibrotic effect in fibrotic liver; so it is difficult to select the drug that would be targeted for delivery to KC and its proper therapeutic opportunity.

9. Conclusions

Several kinds of targeted carrier have been designed and explored in the treatment for liver fibrosis. Most of these carriers may be preferentially taken up by HSC *in vivo*, especially activated HSC, which enable antifibrotic agents carried by them to show a prominent antifibrotic effect with less off-target adverse effect in animal models of liver fibrosis. Drug targeting preparations may create new leads for new therapeutic interventions. As a consequence, this may provide a new pathway for the treatment of liver fibrosis.

10. Expert opinion

Targeted delivery systems have shown an attractive prospect and opened a door for the treatment of liver fibrosis. With their help, many drugs that could not accumulate around the target cells with effective concentration *in vivo* may show a significant antifibrotic effect with fewer adverse events, and the gene therapy for liver fibrosis may show a targeted interference effect to the targeting hepatic cells. Among these targeted carriers, small peptides such as cyclic RGD

peptides and PDGF receptor-recognizing peptides have the advantage of being chemically defined and can be manufactured in large quantities and at high purity without biological contaminants [17].

It should be considered, however, whether targeting of drugs to just one hepatic cell type will be sufficient to delay or even reverse the fibrotic process. An adequate treatment of a complex disease such as liver fibrosis may necessitate a combination of drug targeting preparations to achieve an alternative for the liver transplantation. This means, for example, the targeting of the proliferation inhibitor to HSC in combination with the targeting of the anti-inflammatory drug to KC and/or LEC. In addition, all of the *in vivo* research on these targeted carriers has been performed in animal models, so it is necessary to explore further whether these carriers could also show the same targeted effect in humans with liver fibrosis or cirrhosis. Before doing this, research should be completed on the pharmacokinetic and the long-term adverse effects of these carriers, such as an immune response, in particular, after multiple injections of the carrier preparation, which may constitute a major drawback for future therapeutic applications.

Drug targeting also offers possibilities in the research areas for the pathogenesis of liver fibrosis. Targeting of pharmacological active agents or interference genes to an individual cell type offers the advantage of selective elimination of one cell type or blockade of a single process within this cell type. After specific inhibition of a process, the implications for the development of liver fibrosis can be studied. In this way, drug targeting allows us to gain more insight into the molecular basis of liver fibrosis *in vivo* [16].

In addition, these targeted delivery systems could be used to deliver diagnostic or imaging molecules for assessment of liver fibrosis, currently a major unmet clinical need [98]. The prognosis and management of chronic liver diseases often depend strongly on the degree of liver fibrosis. This is particularly true of chronic hepatitis C virus infection. Until recently, liver biopsy examination was the only gold standard for evaluating liver fibrosis. However, liver biopsy examination is invasive and painful, and can have life-threatening complications. The poor acceptability of liver biopsy examination can lead to treatment delays, and liver biopsy examination is difficult to repeat in poorly symptomatic subjects. The accuracy of liver biopsy examination for assessing fibrosis also has been questioned because of sampling errors and intra- and interobserver variability that may lead to over- or understaging of fibrosis. There is thus a need for accurate non-invasive methods of measuring the degree of liver fibrosis [99]. With the help of targeted carriers, diagnostic or imaging molecules could be accumulated around HSC that are activated and proliferative in liver fibrogenesis. So the degree of liver fibrosis could be shown indirectly. This research has been carried out in the authors' laboratory.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Day CP. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? Gut 2002;50:585-8
2. Iredale JP. Cirrhosis: new research provides a basis for rational and targeted treatments. BMJ 2003;327:143-7
3. Friedman SL. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. N Engl J Med 1993;328:1828-35
4. Fallowfield JA, Iredale JP. Reversal of liver fibrosis and cirrhosis–an emerging reality. Scott Med J 2004;49:3-6
5. Beljaars L, Molema G, Weert B, et al. Albumin modified with mannose 6-Phosphate: a potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatology 1999;29:1486-93
6. Friedman SL. Liver fibrosis–from bench to bedside. J Hepatol 2003;38:S38-53
7. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-18
8. Maher JJ. Interactions between hepatic stellate cells and the immune system. Semin Liver Dis 2001;21:417-26
9. Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. Semin Liver Dis 2001;21:397-416
10. Reynaert H, Thompson MG, Thomas T, Geerts A. Hepatic stellate cells: role in microcirculation and pathophysiology of portal hypertension. Gut 2002;50:571-81
11. Pinzani M, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. J Hepatol 2005;42:S22-36
12. Beljaars L, Poelstra K, Molema G, Meijer DKF. Targeting sugar- and charge-modified albumins to fibrotic rat livers: the accessibility of hepatic cells after chronic bile duct ligation. J Hepatol 1998;29:579-88
13. Torchilin VP. Drug targeting. Eur J Pharm Sci 2000;11:S81-91
14. Beljaars L, Meijer DK, Poelstra K. Targeting hepatic stellate cells for cell-specific treatment of liver fibrosis. Front Biosci 2002;7:e214-22
15. Elrick LJ, Leel V, Blaylock MG, et al. Generation of a monoclonal human single chain antibody fragment to hepatic stellate cells – a potential mechanism for targeting liver anti-fibrotic therapeutics. J Hepatol 2005;42:888-96
16. Beljaars L, Molema G, Schuppan D, et al. Successful targeting to rat hepatic stellate cells using albumin modified with cyclic peptides that recognize the collagen type VI receptor. J Biol Chem 2000;275:12743-51
17. Aina OH, Marik J, Liu R, et al. Identification of novel targeting peptides for human ovarian cancer cells using 'one-bead one-compound' combinatorial libraries. Mol Cancer Ther 2005;4:806-13
18. De Bleser PJ, Scott CD, Niki T, et al. Insulin-like growth factor II/mannose 6-phosphate-receptor expression in liver and serum during acute CCl4 intoxication in the rat. Hepatology 1996;23:1530-7
19. Scharf JG, Knittel T, Dombrowski F, et al. Characterization of the IGF axis components in isolated rat hepatic stellate cells. Hepatology 1998;27:1275-84
20. Kornfeld S. Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. Annu Rev Biochem 1992;61:307-30
21. Beljaars L, Olinga P, Molema G, et al. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P28-HSA). Liver 2001;21:320-8
22. Schneiderhan W, Schmid-Kotsas A, Zhao J, et al. Oxidized low-density lipoproteins bind to the scavenger receptor, CD36, of hepatic stellate cells and stimulate extracellular matrix synthesis. Hepatology 2001;34:729-37
23. Adrian JE, Poelstra K, Scherphof GL, et al. Interaction of targeted liposomes with primary cultured hepatic stellate cells: involvement of multiple receptor systems. J Hepatol 2006;44:560-7
24. Kamps JA, Morselt HW, Swart PJ, et al. Massive targeting of liposomes, surface-modified with anionized albumins, to hepatic endothelial cells. Proc Natl Acad Sci USA 1997;94:11681-5
25. Dahms NM, Hancock MK. P-type lectins. Biochim Biophys Acta 2002;1572:317-40
26. Hughes DA, Fraser IP, Gordon S. Murine macrophage scavenger receptor: in vivo expression and function as receptor for macrophage adhesion in lymphoid and non-lymphoid organs. Eur J Immunol 1995;25:466-73
27. Pitas RE, Friera A, McGuire J, Dejager S. Further characterization of the acetyl LDL (scavenger) receptor expressed by rabbit smooth muscle cells and fibroblasts. Arterioscler Thromb 1992;12:1235-44
28. Schuppan D, Strobel D, Hahn EG. Hepatic fibrosis – therapeutic strategies. Digestion 1998;59:385-90
29. Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. Physiol Rev 1997;77:759-803
30. Luk JM, Zhang QS, Lee NP, et al. Hepatic stellate cell-targeted delivery of M6P-HSA-glycyrrhetinic acid attenuates hepatic fibrogenesis in a bile duct ligation rat model. Liver Intern 2007;27:548-57
31. Hagens WI, Beljaars L, Mann DA, et al. Cellular targeting of the apoptosis-inducing compound gliotoxin to fibrotic rat livers. J Pharmacol Exp Ther 2008;324:902-10
32. Gonzalo T, Talman EG, Van de Ven A, et al. Selective targeting of pentoxifylline to hepatic stellate cells using a novel platinum-based linker technology. J Control Release 2006;111:193-203
33. Gonzalo T, Bataller R, Sancho-Bru P, et al. Short-term treatment with losartan targeted to activated stellate cells reduces advanced liver fibrogenesis: a new strategy to treat liver fibrosis. Hepatology 2005;42:604A
34. Greupink R, Bakker HI, Bouma W, et al. 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 2006;317:514-21
35. Ueki T, Kaneda Y, Tsutsui H, et al. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat Med 1999;5:226-30
36. Isaka Y, Tsujie M, Ando Y, et al. Transforming growth factor-beta 1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction. Kidney Int 2000;58:1885-92
37. Adrian JE, Kamps JA, Poelstra K, et al. Delivery of viral vectors to hepatic stellate cells in fibrotic livers using HVJ envelopes fused with targeted liposomes. J Drug Target 2007;15:75-82
38. Adrian JE, Poelstra K, Scherphof GL, et al. 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 2007;321:536-43

39. Ruoslahti E. RGD and other recognition sequences for integrins. Annu Rev Cell Dev Biol 1996;12:697-715

40. Aumailley M, Gayraud B. Structure and biological activity of the extracellular matrix. J Mol Med 1998;76:253-65

41. Loreal O, Clement B, Schuppan D, et al. Distribution and cellular origin of collagen VI during development and in cirrhosis. Gastroenterology 1992;102:980-7

42. Takahara T, Sollberg S, Muona P, Uitto J. Type VI collagen gene expression in experimental liver fibrosis: quantitation and spatial distribution of mRNAs, and immunodetection of the protein. Liver 1995;15:78-86

43. Marcelino J, McDevitt CA. Attachment of articular cartilage chondrocytes to the tissue form of type VI collagen. Biochim Biophys Acta 1995;1249:180-8

44. Meijer DKF, Beljaars L, Molema G, Poelstra K. Disease-induced drug targeting using novel peptide-ligand albumins. J Control Release 2001;72:157-64

45. Du SL, Pan H, Lu WY, et al. Cyclic Arg-Gly-Asp peptide labeled liposomes for targeting drug therapy of hepatic fibrosis in rats. J Pharmacol Exp Ther 2007;322:560-8

46. Li F, Sun JY, Wang JY, et al. Effect of hepatocyte growth factor encapsulated in targeted liposomes on liver cirrhosis. J Control Release 2008;131:77-82

47. Edelmann L, Hanson PI, Chapman ER, Jahn R. Synaptobrevin binding to synaptophysin: a potential mechanism for controlling the exocytotic fusion machine. EMBO J 1995;14:224-31

48. Kalina M, Lukinius A, Grimelius L, et al. Ultrastructural localization of synaptophysin to the secretory granules of normal glucagon and insulin cells in human islets of Langerhans. Ultrastruct Pathol 1991;15:215-9

49. Gould VE, Lee I, Warren WH. Immunohistochemical evaluation of neuroendocrine cells and neoplasms of the lung. Pathol Res Pract 1988;183:200-13

50. Oberg K. Neuroendocrine gastrointestinal tumours. Ann Oncol 1996;7:453-63

51. Poola I, Graziano SL. Expression of neuron-specific enolase, chromogranin A, synaptophysin and Leu-7 in lung cancer cell lines. J Exp Clin Cancer Res 1998;17:165-73

52. Evans GJ. Tyrosine phosphorylation of synaptophysin in synaptic vesicle recycling. Biochem Soc Trans 2005;33:1350-3

53. Cassiman D, van Pelt J, De Vos R, et al. Synaptophysin: a novel marker for human and rat hepatic stellate cells. Am J Pathol 1999;155:1831-9

54. Elrick LJ, Leel V, Blaylock MG, et al. Generation of a monoclonal human single chain antibody fragment to hepatic stellate cells – a potential mechanism for targeting liver anti-fibrotic therapeutics. J Hepatol 2005;42:888-96

55. Douglass A, Wallace K, Parr R, et al. Antibody-targeted myofibroblast apoptosis reduces fibrosis during sustained liver injury. J Hepatol 2008;49:88-98

56. Powell DW, Mifflin RC, Valentich JD, et al. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol 1999;277:C1-9

57. Heldin CH. Simultaneous induction of stimulatory and inhibitory signals by PDGF. FEBS Lett 1997;410:17-21

58. Claesson-Welsh L. Signal transduction by the PDGF receptors. Prog Growth Factor Res 1994;5:37-54

59. Friedman SL. The virtuosity of hepatic stellate cells. Gastroenterology 1999;117:1244-6

60. Win KM, Charlotte F, Mallat A, et al. Mitogenic effect of transforming growth factor-beta 1 on human Ito cells in culture—evidence for mediation by endogenous platelet-derived growth factor. Hepatology 1993;18:137-45

61. Wong L, Yamasaki G, Johnson RJ, Friedman SL. Induction of β platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture. J Clin Invest 1994;94:1563-9

62. Pinzani M, Milani S, Herbst H, et al. Expression of platelet-derived growth factor and its receptors in normal human liver and during active hepatic fibrogenesis. Am J Pathol 1996;148:785-800

63. Clements JM, Bawden LJ, Bloxidge RE, et al. Two PDGF-B chain residues, arginine 27 and isoleucine 30, mediate receptor binding and activation. EMBO J 1991;10:4113-20

64. Beljaars L, Weert B, Geerts A, et al. The preferential homing of a platelet derived growth factor receptor-recognizing macromolecule to fibroblast-like cells in fibrotic tissue. Biochem Pharmacol 2003;66:1307-17

65. Fretto LJ, Snape AJ, Tominson JE, et al. Mechanism of platelet-derived growth factor (PDGF) AA, AB, and BB binding to alpha and beta receptor. J Biol Chem 1993;268:3625-31

66. Li L, Tao J, Davaille J, et al. 15-deoxy-Delta 12,14-prostaglandin J2 induces apoptosis of human hepatic myofibroblasts. A pathway involving oxidative stress independently of peroxisome-proliferator- activated receptors. J Biol Chem 2001;276:38152-8

67. Li L, Grenard P, Nhieu JT, et al. Heme oxygenase-1 is an antifibrogenic protein in human hepatic myofibroblasts. Gastroenterology 2003;125:460-9

68. Miyahara T, Schrum L, Rippe R, et al. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000;275:35715-22

69. Cawello W, Schweer H, Muller R, et al. Metabolism and pharmacokinetics of prostaglandin E(1) administered by intravenous-infusion in human subjects. Eur J Clin Pharmacol 1994;46:275-7

70. Hagens WI, Mattos A, Greupink R, et al. Targeting 15d-prostaglandin J2 to hepatic stellate cells: two options evaluated. Pharm Res 2007;24:566-74

71. Blomhoff R, Wake K. Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J 1991;5:271-7

72. Fortuna VA, Martucci RB, Trugo LC, Borojevic R. Hepatic stellate cells uptake of retinol associated with retinol-binding protein or with bovine serum albumin. J Cell Biochem 2003;90:792-805

73. Sato Y, Murase K, Kato J, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat biotechnol 2008;26:431-42

•• **This article was designed with a businesslike procedure to demonstrate the targeted effect of vitamin A-coupled liposomes in fibrotic liver, which may be considered as a typical article.**

74. Sauk JJ, Smith T, Norris K, Ferreira L. Hsp47 and the translation-translocation machinery cooperate in the production of alpha 1(I) chains of type I procollagen. J Biol Chem 1994;269:3941-6

75. Nagata K. Expression and function of heat shock protein 47: a collagen-specific

molecular chaperone in the endoplasmic reticulum. Matrix Biol 1998;16:379-86

76. Rockey DC. The cell and molecular biology of hepatic fibrogenesis: clinical and therapeutic implications. Clin Liver Dis 2000;4:319-55
77. Fujimoto J, Kaneda Y. Reversing liver cirrhosis: impact of gene therapy for liver cirrhosis. Gene Ther 1999;6:305-6
78. Monahan PE, Salmuski RJ. AAV vectors: is clinical success on the horizon? Gene Ther 2000;7:24-30
79. Summerford C, Samulski RJ. Membrane-associated heparin sulfate proteoglycan is a receptor for adeno-associated virus type 2 virons. J Virol 1998;72:1438-45
80. Summerford C, Bartlett J, Samulski RJ. αVβ5 integrin: a co-receptor for adeno-associated virus type 2 infection. Nat Med 1999;5:78-82
81. Qing K, Mah C, Hansen J, et al. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adenoassociated virus 2. Nat Med 1999;5:71-7
82. Tsui TY, Lau CK, Ma J, et al. rAAV-mediated stable expression of heme oxygenase-1 in stellate cells: a new approach to attenuate liver fibrosis in rats. Hepatology 2005;42:335-42
83. Bioulac-Sage P, Kuiper J, Van Berkel TJ, Balabaud C. Lymphocyte and macrophage populations in the liver. Hepatogastroenterology 1996;43:4-14
84. Adams DH. Lymphocyte-endothelial cell interactions in hepatic inflammation. Hepatogastroenterology 1996;43:32-43
85. Bijsterbosch MK, Manoharan M, Rump ET, et al. In vivo fate of phosphorothioate antisense oligodeoxynucleotides: predominant uptake by scavenger receptors on endothelial liver cells. Nucleic Acids Res 1997;25:3290-6
86. Schnitzer JE, Bravo J. High affinity binding, endocytosis, and degradation of informationally modified albumins. Potential role of gp30 and gp18 as novel scavenger receptors. J Biol Chem 1993;268:7562-70
87. Yoshioka T, Yamamoto K, Kobashi H, et al. Receptor-mediated endocytosis of chemically modified albumins by sinusoidal endothelial cells and Kupffer cells in rat and human liver. Liver 1994;14:129-37
88. De Rijke YB, Biessen EA, Vogelezang CJ, Van Berkel TJ. Binding characteristics of scavenger receptors on liver endothelial and Kupffer cells for modified low-density lipoproteins. Biochem J 1994;304:69-73
89. Melgert BN, Wartna E, Lebbe C, et al. Targeting of naproxen covalently linked to HSA to sinusoidal cell types of the liver. J Drug Target 1998;5:329-42
90. Kamps JA, Morselt HW, Swart PJ, et al. Massive targeting of liposomes, surface-modified with anionized albumins, to hepatic endothelial cells. Proc Natl Acad Sci USA 1997;94:11681-5
91. Jansen RW, Olinga P, Harms G, Meijer DK. Pharmacokinetic analysis and cellular distribution of the anti-HIV compound succinylated human serum albumin (Suc-HSA) in vivo and in the isolated perfused rat liver. Pharm Res 1993;10:1611-4
92. Franssen EJ, Jansen RW, Vaalburg M, Meijer DK. Hepatic and intrahepatic targeting of an anti-inflammatory agent with human serum albumin and neoglycoproteins as carrier molecules. Biochem Pharmacol 1993;45:1215-26
93. Jansen RW, Molema G, Ching TL, et al. Hepatic endocytosis of various types of mannose-terminated albumins. What is important, sugar recognition, net charge or the combination of these features. J Biol Chem 1991;266:3343-8
94. Beljaars L, Poelstra K, Molema G, Meijer DK. Targeting sugar- and charge-modified albumins to fibrotic rat livers: the accessibility of hepatic cells after chronic bile duct ligation. J Hepatol 1998;29:579-88
95. Melgert BN, Olinga P, Jack VK, et al. Dexamethasone coupled to albumin is selectively taken up by rat nonparenchymal liver cells and attenuates LPS-induced activation of hepatic cells. J Hepatol 2000;32:603-11
96. Melgert BN, Weert B, Schellekens H, et al. The pharmacokinetic and biological activity profile of dexamethasone targeted to sinusoidal endothelial and kupffer cells. J Drug Target 2003;11:1-0
97. Duffield JS, Forbes SJ, Constandinou CM, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56-65
98. Friedman SL. Targeting siRNA to arrear fibrosis. Nat Biotechnol 2008;26:399-40
99. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-50

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