

University of Groningen

Addressing liver fibrosis with lipid-based drug carriers targeted to hepatic stellate cells

Adrian, Joanna Ewa

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Adrian, J. E. (2006). *Addressing liver fibrosis with lipid-based drug carriers targeted to hepatic stellate cells*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

chapter 6



summarizing discussion and perspectives

Summarizing discussion

The research presented in this thesis focuses on the characterisation of liposomes targeted to hepatic stellate cells (HSC) and their potential use as drug carriers for the treatment of liver fibrosis. In the fibrotic liver, HSC are a major source of extracellular matrix (ECM) constituents. Prolonged activation of HSC and deposition of ECM leads ultimately to liver failure (1). Because of the increasing number of patients with liver diseases which often lead to fibrosis and the lack of an effective therapy, alternative treatments for liver fibrosis are urgently needed. Selective targeting of antifibrotic compounds employing cell specific drug carriers to HSC may be essential to the design of an effective therapy for this disease.

In our approach, we applied liposomes as drug carriers. These highly versatile nanovesicles made of phospholipids, are known to be non-toxic, non-immunogenic and biodegradable. Importantly, liposomal drugs are already successfully used in patients, proving their safety in humans (2). However, to ensure specific accumulation of liposomes in HSC in the fibrotic liver, modification of the liposomal surface is required. It was demonstrated that human serum albumin modified with mannose 6-phosphate groups (M6P-HSA) is recognised by HSC in fibrotic livers (3). Particularly, mannose 6-phosphate/insulin-like growth factor II receptor, whose expression is up-regulated on the surface of activated HSC (4), was demonstrated to be the binding receptor for M6P-HSA (5). Based on this assumption, we reasoned that coupling of M6P-HSA to the surface of liposomes will generate a carrier system with a high drug loading capacity that can be specifically targeted to activated HSC in the fibrotic livers.

In **chapter 2** of this thesis, we describe effective coupling M6P-HSA to the surface of liposomes, yielding particles of approximately 100 nm in diameter. This diameter is important because to reach HSC in the liver liposomes have to pass the fenestrations in the endothelial lining which have diameters ranging between 150 and 175 nm (6). The results presented in this chapter demonstrate the accumulation of M6P-HSA liposomes in HSC in the fibrotic livers of bile duct ligated (BDL) rats, which have developed extensive liver fibrosis.

Liposomes with attached M6P-HSA show higher association with cultured HSC than plain liposomes and the uptake of M6P-HSA liposomes by these cells occurs through receptor mediated endocytosis. Interestingly, high association of M6P-HSA liposomes was also observed with quiescent HSC, although these cells do not express the M6P/IGF II receptor. This suggests that, in addition to the M6P/IGF II receptor, there is another kind of receptor that recognises M6P-HSA liposomes. Because the coupling of M6P groups introduces negative charges in the albumin molecule, it is likely that M6P-HSA binds to scavenger receptors, which are known to recognise a variety of polyanionic macromolecules (7). In competition experiments, performed on cultured liver endothelial cells that are known to express scavenger receptors and on HSC, inhibitors of the scavenger receptor did inhibit the association of liposomes modified with M6P-HSA or with polyanionic HSA (aconitylated HSA), confirming the recognition of M6P-HSA liposomes by these receptors. These data indicate that also scavenger receptors are present on the HSC membrane.

The experiments described in **chapter 3** focus on the characterisation of M6P-HSA liposome properties in a rat BDL model of liver fibrosis. Liposomes surface-grafted with M6P-HSA are rapidly cleared from the circulation of BDL rats and accumulate predominantly in the liver. The blood elimination of M6P-HSA liposomes can be inhibited by

M6P-HSA, but not by HSA alone, indicating that the observed rapid clearance is M6P-HSA specific. Identification of the types of liver cells participating in the uptake of M6P-HSA liposomes revealed that, besides HSC, Kupffer cells and liver endothelial cells participate in the uptake of these liposomes. In addition, polyinosinic acid, a competitive inhibitor of scavenger receptors, inhibited both blood elimination of M6P-HSA liposomes and their accumulation in the Kupffer cells and endothelial cells. These results are consistent with the well-established expression of scavenger receptors on Kupffer cells and endothelial cells. In our study, we demonstrate by Western blot and PCR analysis that also HSC express class A scavenger receptors. In activated HSC, expression of the receptor at the protein level is diminished, however.

Results presented in **chapter 2** and **3** provide evidence that more than one receptor system is involved in the recognition of M6P-HSA liposomes, most likely M6P/IGF II receptors and scavenger receptors. As a consequence, the selectivity of M6P-HSA liposomes is not limited to HSC alone.

Chapter 4 describes effects of liposomes containing a bioactive lipid, dilinoleoylphosphatidylcholine (DLPC) in cultured HSC and in livers of BDL rats. DLPC was shown to inhibit activation of HSC and reduce the progression of fibrosis in experimental models of this disease (8;9). In our approach we incorporated DLPC in the membrane of M6P-HSA liposomes, what would give possibility to exploit the specific potential of liposomes to encapsulate more than one compound. By encapsulating an established antifibrotic drug such as troglitazone or roglitazone, DLPC-containing liposomes could provide a dual antifibrotic effect. The liposomal encapsulation of these potent anti-inflammatory and antifibrotic drugs, is expected to keep them efficiently away from the hepatocytes, to which they are toxic. In this way the DLPC liposomes, in concerted action with the encapsulated drugs, may effectively reduce inflammation and fibrosis in the liver (10;11). Our *in vitro* studies demonstrated that both M6P-HSA DLPC-containing liposomes and plain DLPC-containing liposomes have antifibrotic properties. They decreased the expression of profibrotic genes such as collagen I, α -SMA and TGF- β in HSC. However, *in vivo* studies with DLPC-containing liposomes, using BDL rats as a fibrosis model, did not show a similarly clear antifibrotic activity. Only in animals treated with untargeted DLPC-containing liposomes TGF- β and TNF- α were significantly down-regulated at the mRNA level. In the livers of these animals we also observed a decrease of collagen deposition and α -SMA proteins. However, DLPC-containing liposomes modified with M6P-HSA did not show antifibrotic effects in the livers of BDL rats to the extent that was observed in cultured HSC. In contrast, we even observed pro-inflammatory effects in these livers. Incubations of DLPC-containing liposomes with cultured Kupffer cells and liver endothelial cells indicate that DLPC liposomes modulate inflammatory genes such as IL-6, TNF- α and MCP-1 in these cells. Most likely the effects of DLPC containing liposomes in the fibrotic liver are influenced by the *in vivo* accumulation of these liposomes in Kupffer cells and endothelial cells, which are both important regulatory cells in inflammatory responses. Interestingly, DLPC containing liposomes strongly promote the storage of glycogen in hepatocytes of fibrotic livers. During fibrosis, loss of hepatocytes and the impaired functionality of those that are still present are well known characteristics of the disease. Damaged hepatocytes in the fibrotic liver have been shown to lose cytoplasmic glycogen (12;13). The improved glycogen storage observed in BDL rats may indicate that DLPC

containing liposomes have hepatoprotective properties and modulate activities of the non-parenchymal cells.

Although in this *in vivo* study we did not observe a significant regression of fibrosis after treatment with DLPC-containing liposomes, the obtained results justify further investigation of the effects such liposomes may have on progression of the disease. Studies with multiple injections of DLPC-containing liposomes might show more pronounced antifibrotic effects. However, as the bile duct ligated model of liver fibrosis used in these studies is characterised by rapid irreversible development of severe fibrosis, a different animal model of liver fibrosis, such as the CCl₄ model, might be more suitable for the assessment of therapeutic effects.

Specificity of M6P-HSA liposomes for HSC is an important issue for the application of these liposomes as drug carriers. The recognition of M6P-HSA by scavenger receptors causes accumulation of M6P-HSA liposomes in Kupffer cells and endothelial cells. This fact has to be taken into account when the drug to be encapsulated in M6P-HSA liposomes is chosen. For example, compounds which induce apoptosis would likely enhance fibrosis by causing death of these three populations of liver cells, while inhibitors of ECM production, which mainly affect HSC, might attenuate the disease.

Alternatively, instead of M6P-HSA another ligand, characterised by a higher selectivity for HSC, can be coupled to the surface of liposomes. In this respect, human serum albumin modified with a cyclic peptide recognising either the PDGF receptor or collagen VI receptor on HSC might be an option (14;15).

More advanced quantitative methods for evaluating the uptake of M6P-HSA liposomes or other carriers systems such as modified HSA, by the different cell populations in diseased livers, are crucial for the further development of targeted drug carriers for the treatment of liver fibrosis. Immunohistochemical analysis provides only qualitative or semi-quantitative data which are not appropriate to estimate the concentrations of the carrier and/or drug in HSC and other major cell populations of the liver like Kupffer cells, endothelial cells and hepatocytes. As a result, the effects of an applied treatment using drug carriers are difficult to evaluate. Mechanisms underlying the *in vivo* interaction of drug carriers with cells in a disease as complex as liver fibrosis are not easy to elucidate but understanding these mechanisms is pivotal to the improvement and development of drug-carrier mediated therapies. Isolation and purification of the different cell types involved in this disease from the fibrotic liver is an option for quantitative determination of drug carrier uptake. However, because of excessive ECM deposition, the isolation of pure cell populations is difficult, and therefore established isolation methods must be adapted to the fibrotic condition of the livers.

In **chapter 5**, M6P-HSA liposomes were used to prepare targeted particles containing the Hemagglutinating Virus of Japan (HVJ) for the delivery of therapeutic genes or antisense oligonucleotides to HSC. HVJ vectors were shown to have high transfection efficiency *in vitro* and *in vivo* (16), yet uptake of these vectors in HSC is not possible without targeting. Fusion with the cell membrane is the mechanism by which HVJ infects cells. It was also demonstrated that HVJ are able to fuse with liposomes. We incorporated a plasmid containing a reporter gene into inactivated HVJ. Subsequently, these HVJ envelopes were fused with M6P-HSA liposomes. The particles obtained in this fusion were characterised and their ability to target HSC in the fibrotic liver was determined. The data indicate that newly formed particles resulting from the fusion of HVJ envelopes and M6P-HSA

liposomes are targeted to HSC in the fibrotic liver. However, the transfection efficiency of M6P-HSA-HVJ-liposomes in an in vitro test system was low. The reduced transfection efficiency might be due to the loss of plasmid DNA during the fusion process and/or to the redirection of the particle to the M6P/IGF II receptor. Additional experiments are required to elucidate the precise mechanism of transfection using the M6P-HSA-HVJ liposomes. Future studies along these lines might ultimately provide a suitable vehicle for the delivery of genes or antisense oligonucleotides to HSC using M6P-HSA-HVJ liposomes so as to inhibit the activation of these cells in the fibrotic liver and thus, reduce fibrosis.

In summary, in this thesis we demonstrated that liposomes can exert two functions in the therapy for liver fibrosis. Firstly, targeted liposomes can be used as carriers of antifibrotic compounds to HSC which are crucial cells in the development of fibrosis. Secondly, liposomes containing DLPC may modulate different processes such as inflammation and fibrogenesis in different cell types of the fibrotic liver. Thus, the dual functionality of liposomes as a drug carrier system may be successfully exploited in new approaches to treat liver fibrosis.

References

1. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; 115: 209-18.
2. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science* 2004; 19;303: 1818-22.
3. Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis GM, Meijer DK, Poelstra K. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of anti-fibrotic drugs to rat and human hepatic stellate cells. *Hepatology* 1999; 29: 1486-93.
4. De Bleser PJ, Jannes P, Buul-Offers SC, Hoogerbrugge CM, van Schravendijk CF, Niki T, Rogiers V, van den Brande JL, Wisse E, Geerts A. Insulinlike growth factor-II/mannose 6-phosphate receptor is expressed on CCl4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-beta in cocultures with sinusoidal endothelial cells. *Hepatology* 1995; 21: 1429-37.
5. Beljaars L, Olinga P, Molema G, de Bleser P, Geerts A, Groothuis GM, Meijer DK, Poelstra K. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P(28)-HSA). *Liver* 2001; 21: 320-8.
6. Wisse E, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology* 1985; 5: 683-92.
7. Steinbrecher UP. Receptors for oxidized low density lipoprotein. *Biochim Biophys Acta* 1999; 1436: 279-98.
8. Lieber CS, Robins SJ, Li J, DeCarli LM, Mak KM, Fasulo JM, Leo MA. Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology* 1994; 106: 152-9.
9. Ma X, Zhao J, Lieber CS. Polyethylphos-

phatidylcholine attenuates non-alcoholic hepatic fibrosis and accelerates its regression. *J Hepatol* 1996; 24: 604-13.

10. Galli A, Crabb DW, Ceni E, Salzano R, Mello T, Svegliati-Baroni G, Ridolfi F, Trozzi L, Surrenti C, Casini A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology* 2002; 122: 1924-40.

11. Uchimura K, Nakamuta M, Enjoji M, Irie T, Sugimoto R, Muta T, Iwamoto H, Nawata H. Activation of retinoic X receptor and peroxisome proliferator-activated receptor-gamma inhibits nitric oxide and tumor necrosis factor-alpha production in rat Kupffer cells. *Hepatology* 2001; 33: 91-9.

12. Krahenbuhl L, Lang C, Ludes S, Seiler C, Schafer M, Zimmermann A, Krahenbuhl S. Reduced hepatic glycogen stores in patients with liver cirrhosis. *Liver Int* 2003; 23: 101-9.

13. Krahenbuhl L, Talos C, Reichen J, Krahenbuhl S. Progressive decrease in tissue

glycogen content in rats with long-term cholestasis. *Hepatology* 1996; 24: 902-7.

14. Beljaars L, Weert B, Geerts A, Meijer DK, Poelstra K. 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.

15. Beljaars L, Molema G, Schuppan D, Geerts A, De Bleser PJ, Weert B, Meijer DK, Poelstra K. 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.

16. Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N, Nakamura H, Morishita R, Kotani H. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. *Mol Ther* 2002; 6: 219-26.

Perspectives

Chronic liver disease, primarily caused by factors like hepatitis C or B infection, alcohol abuse or metabolic disorders often leads to fibrosis in this organ. Liver transplantation is the ultimate treatment of advanced fibrosis but because of lack of liver donors, high costs and complexity of this therapy, there is a strong urge to establish alternative treatments. Currently, various strategies are investigated for their efficacy in attenuating fibrosis, but so far none of them has been approved for use in humans. Low specificity of experimental treatments towards particular cells in the liver that are involved in the fibrotic process often hamper the antifibrotic effects *in vivo* and alter the results obtained with this compounds *in vitro*. Therefore, cell specific delivery of drugs to the fibrotic liver using carriers such as liposomes represents an attractive alternative for traditional treatments.

Liposomal drugs available on the market for cancer treatment prove their potential in the therapy of chronic disease. The practical use of liposomes as carriers in liver fibrosis would require separate toxicity and safety studies, but liposomes are in principle suitable for a long-term therapy.

According to current views, HSC producing extracellular matrix constituents are a major target for antifibrotic therapies. In order to deliver liposomes to HSC, selective targeting strategies need to be employed. On the basis of research presented in this thesis we conclude that targeting of liposomes to HSC is feasible. However, the partial uptake of the targeted liposomes by other cells than HSC complicates the applicability of these liposomes. Thus, highly selective liposomal carriers to HSC should be a priority for future research. In addition, methods for quantitative determination of the amount of liposomes and carried compound delivered to the HSC need to be developed.

Liver fibrosis is a complex disease, in which besides ECM deposition other processes like inflammation and oxidative stress occur simultaneously and contribute to the development of fibrosis. Therefore the role of other cell types, such as Kupffer cells and liver endothelial cells should not be ignored. To effectively cure liver fibrosis, the uptake of our carrier system by these cells may be quite relevant as a concerted action of two or more compounds interfering with different pathways in the disease process may be required for optimal effectivity. Targeted liposomal drug carriers have been shown to be able to target all major cell populations of the liver such as hepatocytes, Kupffer cells, liver endothelial cells and now also HSC. This opens the possibility to selectively modulate the action of different cells involved in the fibrotic process. However, future studies using liposomal drugs for treatment of liver fibrosis will have to demonstrate their practical applicability.

*If A equals success, then the formula is: $A = X + Y + Z$.
X is work. Y is play. Z is keep your mouth shut.*

Albert Einstein