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Interaction of targeted liposomes with primary cultured hepatic stellate cells: Involvement of multiple receptor systems

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***Background/Aims*: In designing a versatile liposomal drug carrier to hepatic stellate cells (HSC), the interaction of mannose 6-phosphate human serum albumin (M6P-HSA) liposomes with cultured cells was studied.**

***Methods*: M6P-HSA was covalently coupled to the liposomal surface and the uptake and binding of ^{3}H-labelled M6P-HSA liposomes by primary rat HSC and liver endothelial cells was determined. The targeting ability of M6P-HSA liposomes to HSC was tested in bile duct ligated rats using immunohistochemical methods.**

***Results*: The association of M6P-HSA liposomes with HSC was 4-fold higher than of control liposomes. An excess of M6P-HSA inhibited this association by 58%, indicating M6P receptor specificity. The scavenger receptor competitor polyinosinic acid abolished association of M6P-HSA liposomes with HSC. M6P-HSA liposomes also amply associated with endothelial cells, which abundantly express scavenger receptors. Endocytosis of M6P-HSA liposomes by HSC was temperature dependent and could be inhibited by monensin. In the fibrotic liver M6P-HSA liposomes co-localised with HSC.**

***Conclusions*: Coupling of M6P-HSA to liposomes strongly increases the in vitro uptake of these liposomes by HSC and endothelial cells. Both the mannose 6-phosphate receptor and the scavenger receptors are involved in the uptake process. M6P-HSA liposomes are potential drug carriers to HSC in the fibrotic liver.**

***Keywords*: Hepatic stellate cells; Liposomes; M6P/IGF II receptor; Liver fibrosis; Scavenger receptor**

1. Introduction

In the healthy liver, hepatic stellate cells (HSC) express a quiescent phenotype and are responsible for storage of vitamin A [1]. During liver fibrosis, HSC become activated and transform into proliferating fibroblast-like cells, which produce large amounts of collagen [2]. Since HSC are identified as key players in the development of fibrosis, they are attractive targets for antifibrotic therapy.

Human serum albumins (HSA) modified with sugar moieties or cyclic peptides that recognise receptors on HSC were shown to selectively accumulate in HSC in a rat model of liver fibrosis. In particular, mannose 6-phosphate groups attached to HSA (M6P-HSA) exerted specificity for the mannose 6-phosphate/insulin like growth factor II (M6P/IGF II) receptors present on HSC [3–5]. The expression of these receptors is increased during HSC transformation from the quiescent into the activated phenotype [6,7]. In the fibrotic liver M6P/IGF II receptors facilitate the activation of transforming growth factor β (TGF-β) [8,9] which is a potent cytokine that stimulates the production of collagen by HSC. Binding of latent TGF-β to the M6P/IGF II receptors mediated by two M6P groups on a

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short peptide enables plasmin-mediated cleavage of the peptide and generation of active TGF-β [8,9].

Intracellular M6P receptors are responsible for sorting of lysosomal enzymes [10,11]. Approximately 10–20% of the total number of M6P/IGF II receptors is present on the surface of the cells [12] where they bind diverse M6P- and non M6P-containing ligands such as TGF-β, proliferin, IGF II, retinoic acid and urokinase-type plasminogen activator receptor [13].

In the present study, we coupled M6P-HSA to the surface of liposomes as a homing ligand to HSC. Liposomes have a high capacity for encapsulation of various drugs, but so far this drug delivery system had not been specifically targeted to HSC in fibrotic livers. We performed in vitro studies in primary cultures of HSC and in vivo experiments using a rat model of liver fibrosis to determine whether M6P-HSA modified liposomes specifically associate with HSC. Furthermore, the mechanism of this interaction was characterised. Since modification of HSA by M6P groups introduces negative charges to the protein, we also examined the interaction of M6P-HSA liposomes with primary cultures of liver endothelial cells (LEC) that express scavenger receptors (ScR) [14].

It is conceivable that ScR also play a role in the association of M6P-HSA liposomes with LEC and HSC.

2. Materials and methods

2.1. Materials

Cholesterol (Chol), *N*-succinimidyl-*S*-acetylthioacetate (SATA), cis-aconitic anhydride, monensin and polyinosinic acid (poly I) were from Sigma (St Louis MO, USA). 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[4-(maleimidophenyl)butyramide] (MPB-PE) were purchased from Avanti Polar Lipids (Alabaster AL, USA). [^{3}H]cholesteryloleyl ether (^{3}H-COE) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). Human serum albumin fraction V was from the Central Laboratory of the Red Cross (Amsterdam, The Netherlands). Dulbecco's modified Eagles Medium (DMEM), RPMI-1640, L-glutamine were obtained from Invitrogen (Paisely, Scotland, UK), foetal calf serum (FCS) from BioWhittaker Europe (Verviers, Belgium), penicillin, streptomycin from Sigma. All other chemicals were analytical grade or the best grade available.

2.2. Animals

Specified pathogen free (SPF) male Wistar and Wag/Rij rats (Harlan, Horst, The Netherlands) were housed under standard laboratory conditions with free access to food and water. The local committee for care and use of laboratory animals approved the presented study.

2.3. Preparation of modified HSA

HSA was modified either with mannose 6-phosphate moieties [4] or with cis-aconitic anhydride yielding cis-aconitylated HSA (AcoHSA) [15] as described before.

2.4. Preparation of liposomes

Liposomes were composed of POPC, CHOL, MPB-PE in molar ratio of 23:16:1, labelled with a trace amount of ^{3}H-COE, a non-degradable bilayer marker and prepared as described before [16]. The phospholipid phosphorus content of each liposome preparation was assessed by phosphate assay [17]. The lipid concentration was calculated taking into account the amount of cholesterol in the preparation of liposomes. Size and size distribution were determined by dynamic laser light scattering with a Nicomp submicron particle analyzer (NICOMP 380 ZLS, Santa Barbara, CA, USA). The average diameter of the liposomes was obtained from the volume distribution curves produced by the particle analyzer. M6P-HSA and AcoHSA were subsequently coupled to liposomes by the SATA method described before [15]. The phospholipid phosphorus, protein content [18] and particle size of both M6P-HSA and AcoHSA liposomes were determined. Control liposomes were prepared similarly but they were not coupled with protein. Liposomes were stored under argon at 4 °C and used within three weeks after preparation.

2.5. Isolation of HSC and LEC

HSC were isolated from livers of male Wistar rats (550–600 g) (Harlan) as described before [19]. Isolated HSC were cultured in DMEM containing 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin. One day before the experiments, HSC were trypsinized and seeded in 24 wells plates (Costar).

LEC were isolated from livers of male Wag/Rij rats (200–250 g) (Harlan) as described before [16]. LEC were cultured in RPMI-1640 medium supplemented with 20% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 ng/ml endothelial cell growth factor (Boehringer, Mannheim, Germany) for 2 days in 24 wells collagen-coated plates (Greiner) before being used in the experiments.

2.6. Association of M6P-HSA liposomes and AcoHSA liposomes with HSC and LEC

To study association of M6P-HSA liposomes and AcoHSA liposomes in cultures of HSC or LEC, the cells were pre-incubated for 1 h with FCS-free medium. Next, 3 h incubation with ^{3}H-COE-labelled liposomes (160 nmol/ml, at 37 °C unless stated otherwise) in culture medium without FCS was performed. When appropriate, other agents were added to the incubation mixture as indicated. Incubation was stopped by placing the culture plate on ice and cells were washed with ice-cold PBS pH 7.4. Subsequently, cells were lysed using 0.4 M NaOH. Cell associated radioactivity was determined by liquid scintillation counting of the lysed cell suspension and radioactivity was normalized for protein content measured according to Lowry [20].

2.7. Detection of M6P/IGF II receptor expression by the cells

2.7.1. RNA isolation and reverse-transcription polymerase chain reaction

Total RNA was isolated from HSC and LEC using Absolutely RNA Microprep kit (Stratagene, Amsterdam, The Netherlands) according to the protocol of the manufacturer. Subsequently, synthesis of first strand cDNA from total cellular RNA was performed with SuperScript III Reverse Transcriptase (Invitrogen, Breda, The Netherlands) and 40 units RNaseOut (Invitrogen) in a volume of 20 μl containing 250 ng random primers (Promega). One microliter of cDNA (10 ng/μl) was used for polymerase chain reaction in a total volume of 30 μl containing 1 unit Taq polymerase (*T. aquaticus*; Amersham Biosciences, Roosendaal, The Netherlands) and 10 μM primers. Primers for M6P/IGF II rat receptor were as follows: forward 5′-GTGTCCTCTGGGTGTGGACT-3′, reverse 5′-CTCCTCCTTGCTGACCTTTG-3′ (Sigma Genosys). GAPDH (forward 5′-CGCTGGTGCTGAGTATGTCG -3′, reverse 5′- CTGTGGTCAT-GAGCCCTTCC-3′) (Sigma) was used as house keeping gene. Thirty cycles of reaction for M6P/IGF II receptor primers at 94 °C for 5 min, 94 °C for 30 s, 57 °C for 45 s, 72 °C for 45 s and 72 °C for 7 min, and 26 cycles for

GAPDH primers were carried out using GeneAmp® PCRSystem 9700 (Applied Biosystem). The polymerase chain reaction product was visualized on 1.5% agarose gel containing ethidium bromide.

2.7.2. Immunohistochemical analysis

HSC and LEC were isolated and cultured as described above. After fixation in an acetone: methanol [1:1] solution, cells were incubated with a goat polyclonal antibody against M6P/IGF II receptor (K-21, Santa Cruz Biotechnology, Inc.). Subsequently, endogenous peroxidase was inhibited with H_2O_2, followed by incubation with peroxidase conjugated rabbit-anti-goat IgG (RaGPo, Dako Cytomation, Denmark) in the second step and with peroxidase conjugated goat-anti-rabbit IgG (GaRPo, Dako) in the third. Antibody associated peroxidase was visualised with 3-amino-9-ethyl-carbazole (AEC).

2.8. In vivo accumulation of M6P-HSA liposomes

Liver fibrosis was induced by ligation of the bile duct in male Wistar rats (250 g) under anaesthesia. Three weeks after bile duct ligation (BDL 3), rats developed a severe liver fibrosis and were used for experiments [4]. Two μmol M6P-HSA liposomes per 100 g of body weight were injected into BDL 3 rats via the penile vein. One hour after injection of liposomes, livers were harvested and snap-frozen in isopentane. Cryostat sections of livers (4 μm) were fixed in acetone. Double immunohistochemical staining was performed to assess whether M6P-HSA liposomes were taken up in HSC as described previously [4]. Briefly, M6P-HSA liposomes were stained with a rabbit polyclonal antibody directed against HSA (Cappel, Organon Teknika, Turnhout, Belgium) followed by incubation with mouse monoclonal antibodies directed against desmin (Organon Teknika), an established marker of HSC. Next, the endogenous peroxidase was inhibited with H_2O_2 and the sections were incubated with two secondary antibodies, GaRPo and RaGPo (Dako). The antibody against desmin was detected with alkaline phosphatase conjugated rabbit-anti-mouse IgG (Dako) and visualised with Naphtol AS-MX/Fast Blue reaction at 37 °C. Peroxidase conjugated antibodies were visualised with AEC.

2.9. Statistical analysis

Data were normalized to the control value and presented as mean ± SEM. Statistical significance of differences was evaluated by a two-tailed unpaired Student's *t*-test.

Table 1
Characterization of liposomes

Type of liposomes	Coupled protein (μg protein/μmol TL)	Size (nm)
M6P-HSA liposomes	52 ± 12	101 ± 9
AcoHSA liposomes	47 ± 11	101 ± 6
Control liposomes	–	80 ± 5

Size of liposomes and protein coupled to the liposomes was determined as described in Section 2. Data are presented as a mean ± SD of 6–11 liposome preparations.

3. Results

3.1. Liposome characterization

M6P-HSA used for the preparation of targeted liposomes contained 27 ± 7 molecules of M6P per molecule HSA. In AcoHSA an average of 54 out of the 60 ε-amino groups of HSA groups were aconitylated.

The preparation of M6P-HSA liposomes and AcoHSA liposomes was reproducible and yielded liposomes that were comparable in size and in amount of coupled protein (Table 1).

3.2. Expression of M6P/IGF II receptor on HSC and LEC

Primary rat HSC were used, 3 or 10 days after isolation (referred to as day-3 or day-10 HSC). Day-3 HSC displayed the quiescent phenotype, as assessed with light microscopy. They showed a compact shape and contained lipid droplets. After 10 days of culturing, HSC transformed into an activated cell type, which is characterised by a stretched shape and loss of lipid droplets [21].

The expression of M6P/IGF II receptors by day-3 and day-10 HSC as well by LEC was checked at the mRNA

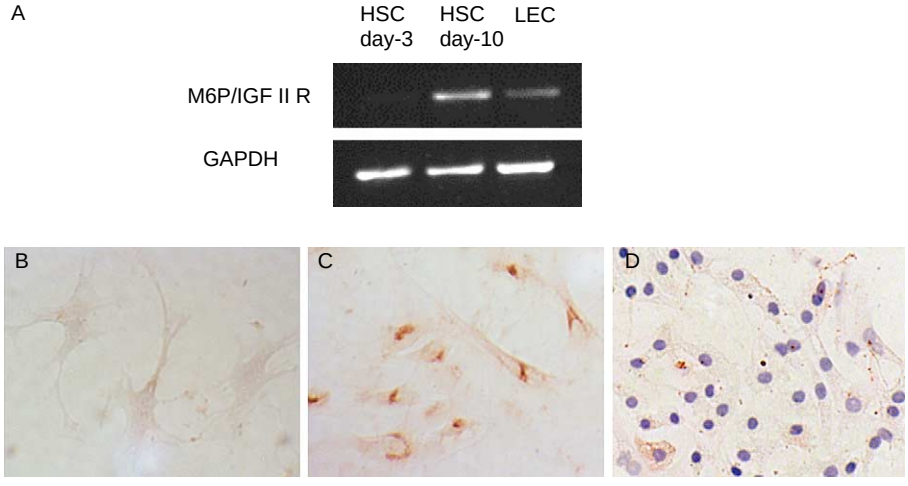

Fig. 1. Expression of M6P/IGF II receptors by HSC and LEC. mRNA expression of M6P/IGF II receptors by day-3 HSC, day-10 HSC and LEC was determined using RT-PCR with GAPDH as a house keeping gene (A). Protein expression of M6P/IGF II receptors by day-3 HSC (B), day-10 HSC (C) and LEC (D) was assessed by immunohistochemical staining for M6P/IGF II receptor. Red cells express M6P/IGF II receptors. At a protein level M6P/IGF II receptor expression could only be detected in day-10 HSC. Original magnification ×200.

level, as well as at the protein level. In day-3 HSC, a relatively low level of M6P/IGF II receptor mRNA (Fig. 1A) was detected, while increased expression was observed in day-10 HSC. These results were confirmed by immunohistochemical staining which revealed higher expression of the M6P/IGF II receptor on day-10 HSC (Fig. 1C) than on day-3 HSC (Fig. 1B). In LEC, the mRNA of M6P/IGF II receptor was also detectable (Fig. 1A), but immunohistochemical staining showed no expression of the receptor at the protein level (Fig. 1D).

3.3. Association of M6P-HSA liposomes with day-3 and day-10 HSC

Coupling M6P-HSA to the surface of liposomes resulted in 5-fold and 3.5-fold higher association with day-3 and day-10 HSC, respectively, when compared to control liposomes (Fig. 2). In absolute amounts, day-3 HSC bound and took up five times as much M6P-HSA liposomes as day-10 cells (Fig. 3).

The specificity of the interaction of M6P-HSA liposomes was assessed by incubating HSC with these liposomes and an excess of free M6P-HSA. M6P-HSA reduced the association with day-3 HSC by 49% ± 8 (Table 2), while a similar effect was observed for day-10 HSC, with an inhibition of 68% ± 5. Unmodified HSA did not inhibit the association of M6P-HSA liposomes, for neither day-3 nor day-10 HSC (data not shown).

3.4. Endocytosis of M6P-HSA liposomes by HSC

The natural ligands for the M6P/IGF II receptor undergo endocytosis after binding to the receptor. In order to investigate whether binding of M6P-HSA liposomes is followed by endocytosis in HSC, the effects of temperature and monensin, a widely used inhibitor of endocytosis were examined [22]. HSC were incubated with M6P-HSA liposomes at 37 and 4 °C. At 4 °C (no internalisation) HSC bound 5-fold less M6P-HSA liposomes than at 37 °C (Fig. 4A). Monensin had no effect on the association of control liposomes, but inhibited the intracellular uptake of M6P-HSA liposomes by 45% ± 6 (Fig. 4B).

3.5. Effect of polyanions on the association of M6P-HSA liposomes and AcoHSA liposomes with HSC and LEC

The discrepancy in the expression of the M6P/IGF II receptors by day-3 and day-10 HSC and the data on the association of M6P-HSA liposomes with these cells suggests that receptors other than M6P/IGF II must also be involved in the binding and uptake of these liposomes. To study whether ScR might mediate this interaction, HSC and LEC were incubated with M6P-HSA liposomes and AcoHSA liposomes in the presence or absence of free M6P-HSA, AcoHSA and polyinosinic acid, by virtue of its polyanionic character a potent antagonist of ScR activity [23,24]. AcoHSA is a

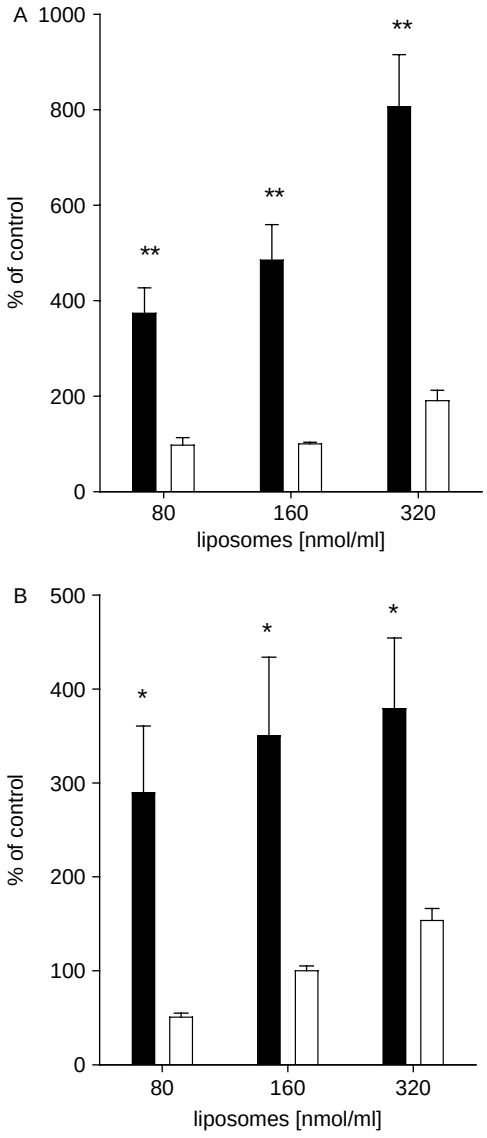

Fig. 2. Association of M6P-HSA liposomes with HSC. HSC 3 days (A) and 10 days (B) after isolation were incubated with 80, 160, 320 nmol/ml ^{3}H-COE labelled M6P-HSA liposomes (closed bars) and control liposomes (open bars). The association of control liposomes at the concentration 160 nmol/ml was taken as 100%. Control values for day-3 HSC and day-10 HSC were 11.7 ± 2.9 and 4.2 ± 0.91 nmol lipid per mg of cell protein, respectively. Data are presented as mean ± SEM of 3–4 experiments. *$P < 0.05$, **$P < 0.001$ versus control liposomes.

highly negatively charged molecule [15], known to be taken up by cells via the ScR [25]. Additionally, LEC express ScR [14], but no M6P receptors (Fig. 1). Association of M6P-HSA liposomes with LEC was 113 nmol lipid/mg protein, while association of AcoHSA liposomes was 69 nmol lipid/mg protein. Furthermore, AcoHSA liposomes associated with day-3 and day-10 HSC (42 and 7 nmol lipid/mg protein, respectively) at comparable levels as M6P-HSA liposomes (56 and 9 lipid/mg protein, respectively) (Fig. 5).

Excess AcoHSA reduced the association of M6P-HSA and AcoHSA liposomes with LEC, day-3 and day-10 HSC stronger than excess of M6P-HSA (Fig. 5). In addition,

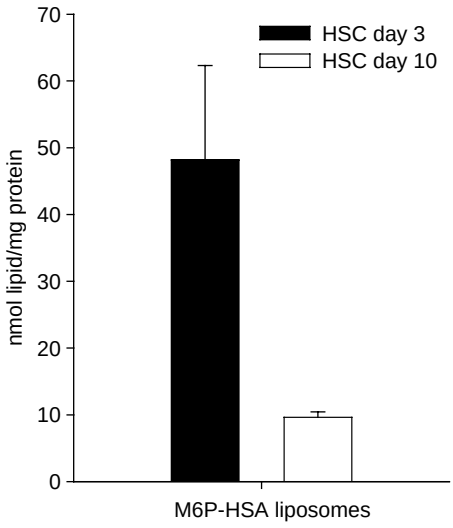

Fig. 3. Comparison of the association of M6P-HSA liposomes with day-3 and day-10 HSC. Cells were incubated with M6P-HSA liposomes at the concentration 160 nmol/ml. Data are presented as mean ± SEM of 3–4 experiments.

polyinosinic acid substantially blocked the uptake of M6P-HSA and AcoHSA liposomes with all types of cells (Fig. 5).

3.6. Uptake of M6P-HSA liposomes by HSC in fibrotic livers

To test the targeting ability of M6P-HSA liposomes to HSC in the fibrotic liver, we injected these liposomes in BDL 3 rats. Accumulation of M6P-HSA liposomes in HSC was determined by a double immunostaining method as described before [4]. M6P-HSA liposomes and HSC were detected simultaneously with specific antibodies against albumin and desmin, respectively. As Fig. 6 shows, double positive cells were found 1 h after injection, indicating accumulation of M6P-HSA liposomes in HSC.

4. Discussion

In the present study, we 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

Table 2
Effect of M6P-HSA on the association of M6P-HSA liposomes with HSC

	M6P-HSA liposomes	M6P-HSA liposomes + M6P-HSA
day-3 HSC	100 ± 2.6	51.0 ± 8.4
day-10 HSC	100 ± 4.0	32.0 ± 4.8

Day-3 and day-10 HSC were incubated with ^{3}H-COE labelled M6P-HSA liposomes in the presence of 0.1 mg/ml M6P-HSA. Data are expressed as % of the control (^{3}H-COE labelled M6P-HSA liposomes). Control values for day-3 and day-10 HSC were 78.2 ± 27.1 and 7.6 ± 1.4 nmol lipid per mg protein, respectively, mean ± SEM of 3 experiments.

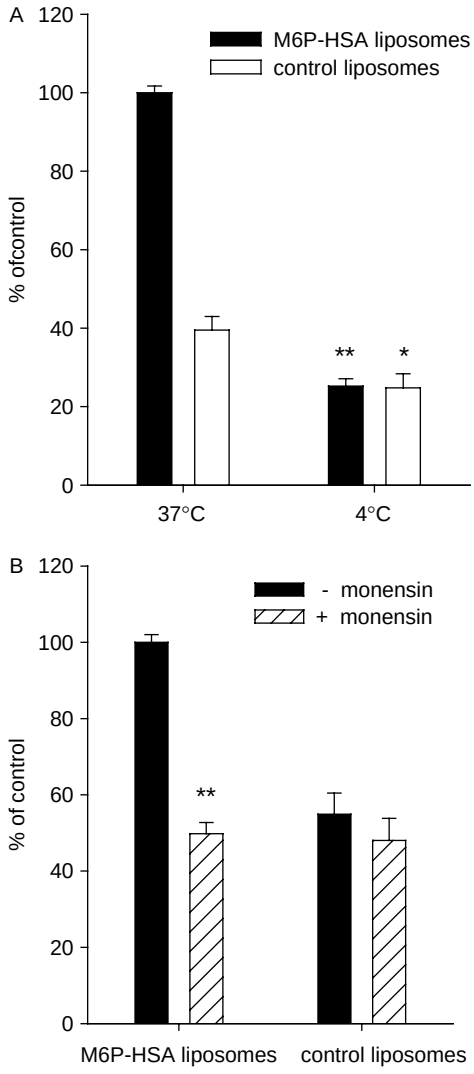

Fig. 4. Effect of temperature and monensin on the association of M6P-HSA liposomes with HSC. Day-10 HSC were incubated with ^{3}H-COE labelled M6P-HSA liposomes at 37 °C or 4 °C (A) and in the presence of monensin (2 μM) (B). Association of M6P-HSA liposomes at 37 °C or without treatment was taken as 100%. Control values for (A) and (B) were 17.6 ± 2.7 and 15 ± 2.5 nmol lipid per mg cell protein, respectively, mean ± SEM of 3–4 experiments. *$P < 0.05$, **$P < 0.001$ versus M6P-HSA liposomes at 37 °C or without treatment.

association of the M6P-HSA liposomes was observed for day-3 HSC than for day-10 HSC, yet the results of RT-PCR and immunohistochemical staining showed abundant presence of the M6P/IGF II receptor on day-10 only. This latter observation is consistent with previous data [7,26], showing that the mRNA and protein levels of the M6P/IGF II receptor increased in HSC cultured for more than 6 days.

We hypothesise, therefore, that two receptor systems might contribute to the recognition of M6P-HSA liposomes. The M6P/IGF II receptors bind M6P-HSA liposomes via M6P groups attached to HSA. The negative charge of M6P-HSA, caused by the clustering of M6P groups on the protein core, could also qualify M6P-HSA liposomes as a ligand for scavenger receptors. Competition studies with free

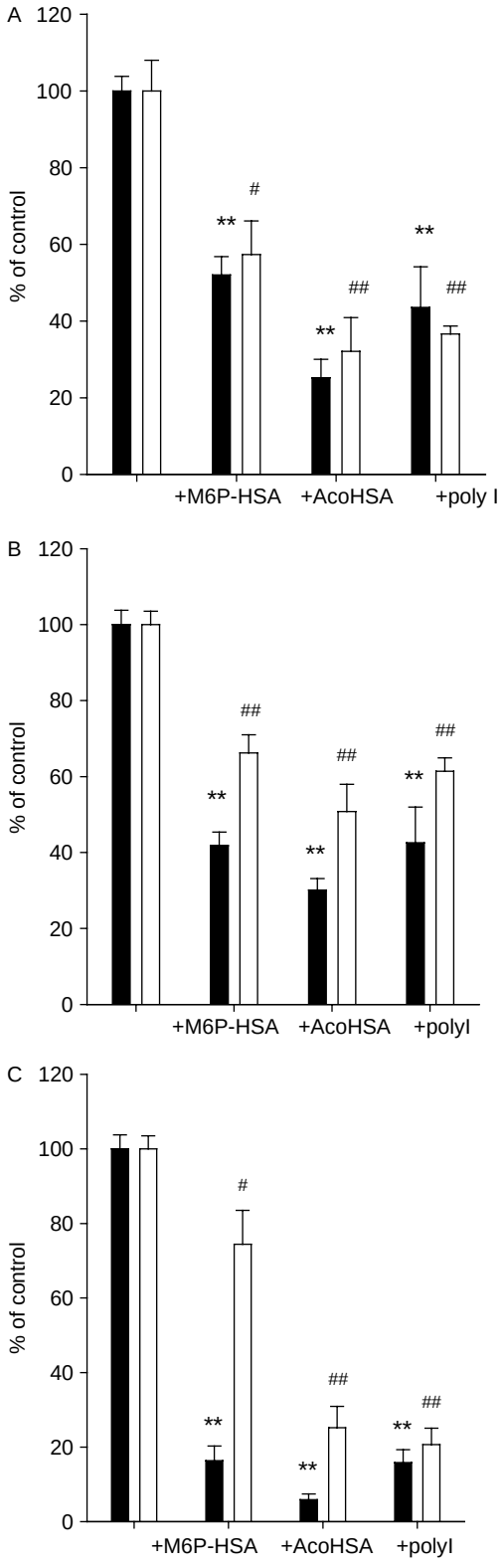

Fig. 5. Comparison of the association of M6P-HSA liposomes and AcoHSA liposomes with HSC and LEC. Day-3 HSC (A), day-10 (B) HSC and LEC (C) were incubated with ^{3}H-COE labelled M6P-HSA liposomes (closed bars) and AcoHSA liposomes (open bars) in the absence or presence of 0.1 mg/ml M6P-HSA, AcoHSA or 10 μg/ml polyinosinic acid. The association of M6P-HSA liposomes and AcoHSA liposomes without treatment was taken as 100%. Control values of M6P-HSA liposomes for day-3 HSC, day-10 HSC and LEC were 56.5±14.9, 8.6±1.9 and 112.6±11.8 nmol lipid per mg protein, respectively. Control values of AcoHSA liposomes for day-3 HSC, day-10 HSC and LEC were 41.6±9.4, 7.4±1.2 and 68.6±8.8 nmol lipid per mg of cell protein, respectively, mean±SEM of 3–4 experiments. *$P<0.05$, **$P<0.001$ versus M6P-HSA liposomes without treatment, $^{\#}P<0.05$, $^{\#\#}P<0.001$ versus AcoHSA liposomes without treatment.

M6P-HSA indicate a specific interaction of M6P-HSA liposomes with both day-3 and day-10 HSC, even though the M6P/IGF II receptor is hardly detectable on day-3 cells. Liposomes coupled with aconitylated HSA have been shown to associate readily with LEC through the ScR [25]. In our studies, AcoHSA liposome association with HSC was comparable to that of M6P-HSA liposomes. Association of M6P-HSA liposomes and AcoHSA liposomes with LEC were both in the same order of magnitude. We did find expression of the M6P/IGF II receptor by LEC at the mRNA, but not at the protein level, which is consistent with data reported earlier [7]. Given the high association of M6P-HSA liposomes with LEC, recognition of M6P-HSA liposomes by ScR seems to be implied. The observed differences in competition between activated and non-activated HSC by M6P-HSA and AcoHSA, respectively, correspond with an increased M6P/IGF II receptor expression on activated HSC. However, to discriminate between the relative contribution of M6P/IGF II receptors and ScR in the uptake of M6P-HSA liposomes by HSC, blocking antibodies against these receptors are required.

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 [27]. In our experiments, though, the inhibitory effect of polyinosinic acid on the uptake of M6P-HSA liposomes and AcoHSA liposomes by HSC suggests that not ScR class B, but rather ScR class A contributes to the association of these liposomes [13]. Although ScR class A is mainly expressed by macrophages, it is also present on endothelial cells, smooth muscle cells and fibroblasts [14,28]. A variety of factors regulate expression of ScR class A in different types of cells; TGF-β and platelet-derived growth factor upregulate ScR class A on smooth muscle cells [29]. In macrophages, interferon gamma and tumor necrosis factor α decrease ScR class A expression [30]. The presence of a wide range of growth factors and cytokines in fibrotic livers, as well as particular stage of activation of the HSC may influence the expression of ScR class A by these cells. Our data suggest that HSC might express the ScR class A, but additional studies will be required to provide direct proof.

We designed M6P-HSA liposomes as a novel drug carrier to HSC, for selective targeting of antifibrotic drugs. Our in vivo data showed that M6P-HSA liposomes are able to reach HSC in the fibrotic liver. In a subsequent study, the in vivo properties of M6P-HSA liposomes as a drug delivery system to HSC will be determined in a rat model of liver

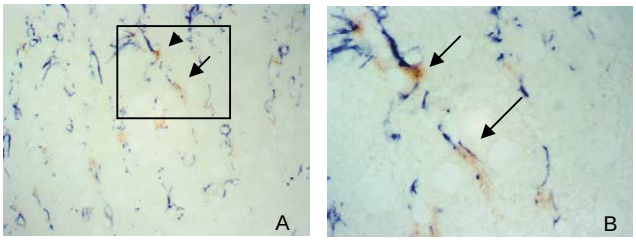

Fig. 6. Accumulation of M6P-HSA liposomes in HSC in the fibrotic liver. Double immunostaining using specific antibodies directed against HSA (red staining) to detect M6P-HSA liposomes and against desmin (blue staining) to identify HSC was performed as described in Materials and Methods. Arrows indicate double positive cells, (A) original magnification ×200, (B) original magnification ×1000 of the selected area from picture (A).

fibrosis, including the contribution of the other liver cell types in the uptake of these liposomes. HSC play a crucial role in the pathogenesis of liver fibrosis, producing large amounts of collagen as well as cytokines and growth factors, all of which perpetuate the fibrotic process in a concerted action [31]. Targeting antifibrotic compounds such as inhibitors of collagen production, to these cells might therefore improve current therapies with cell non-specific drugs.

In conclusion, we showed that coupling M6P-HSA to liposomes strongly enhanced the binding and uptake of these liposomes by cultured primary HSC. In addition, it leads to accumulation of the liposomes in HSC in the fibrotic liver opening possibilities to deliver anti-fibrotic drugs to this cell type. The M6P/IGF II receptor and the ScR mediate the association of M6P-HSA liposomes with HSC. We demonstrated that M6P-HSA liposomes are internalised by these cells, which should enable them to deliver enclosed drugs into the cell. Liposomes targeted to these crucial cells may provide a new means to attenuate fibrotic processes in the liver.

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