

NF-KAPPA B, LIPOSOMES AND PATHOGENESIS OF HEPATIC INJURY AND FIBROSIS

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1. ABSTRACT

The liver injury caused by hepatotoxins is characterized by varying degrees of hepatocyte degeneration and cell death via either apoptosis or necrosis. Generation of reactive intermediate metabolites from the metabolism of toxins and the occurrence of reactive oxygen species (ROS) during the inflammatory reaction account for a variety of pathophysiologic pathways which lead to cell death. This process can then evoke acute or chronic inflammatory responses if the injury is sustained, and these pathologic alterations eventually progress to cirrhosis. Understanding the function of transcription factors, such as nuclear factor kappa B (NF-kappa B), in acute liver injury may provide some answers to the molecular mechanisms of toxic insults. Liposomes have been used as vehicles for drug delivery and gene therapy and they have been shown to have substantial potential in the targeting of specific cell types of the liver. Thus, the use of liposomes may improve targeting efficacy in the treatment of a variety of liver diseases.

2. NF-KAPPA B AND HEPATIC FIBROGENESIS

The pathogenesis of liver injury and fibrosis involves complicated interactions among different cell populations in the liver, soluble factors, such as cytokines, and the extracellular matrix (1). Hepatocytes are injured in a number of pathologic processes (chemical, biological and immunological). In hepatotoxin-induced liver damage, such as ethanol, carbon tetrachloride (CCl₄) and bromobenzene, lipid peroxidation is one of the injurious mechanisms that result from the reactive intermediate metabolites of these hepatotoxins (2). Persistent hepatocellular damage also initiates a healing process—fibrogenesis. Kupffer cells appear to be primarily responsible for mediating the second phase of hepatocellular necrosis, induced by ROS (3,4), and for activating hepatic stellate cells (HSC, Ito cells), the major cell type responsible for enhanced extracellular

matrix production during the fibrogenic process (5). A number of cytokines and other soluble factors, such as tumor necrosis factor-alpha (TNF-alpha), as well as interleukin 2 and 6 (IL-2 and IL-6) participate in the damage of hepatocytes and sinusoidal endothelial cells. Transforming growth factor-beta (TGF-beta) and platelet-derived growth factor (PDGF) have been considered to be fibrogenic cytokines and to contribute to the activation of HSC (6,7). Thus, a number of factors involved in the pathogenesis of hepatic injury and fibrosis are beginning to be categorized.

Our attention has recently been focused on nuclear factor-kappa B (NF-kappa B) as a mediator in the process of liver injury and fibrogenesis (8). This transcriptional factor was first described as a B cell protein that was critical for the transcriptional activation of the kappa light chain enhancer. The binding of NF-kappa B to its DNA consensus sequence triggers transcription of a series of genes. NF-kappa B is a heterodimer of several subunits and is inactivated in the cytoplasm bound to an inhibitory subunit, I-kappa B-alpha. Phosphorylation, initiated by such events as TNF-alpha binding to its receptors, followed by I-kappa B-alpha degradation by proteasomes, triggers the activation of NF-kappa B (9). Recent reports indicate that the I-kappa B-alpha proteolysis is a crucial step, as is the generation of p50 subunits by the processing of a 105 kDa precursor protein (10,11). Following the degradation of I-kappa B-alpha, the activated p50/p65 dimer then translocates to the nucleus and binds to the DNA consensus motif of its many inducible genes. NF-kappa B activity also appears to be regulated transcriptionally (12), and transcriptional regulation may be of some importance during prolonged activation (12). It has become clear that this set of inducible transcription factors (NF-kappa B) is crucial in the cytoplasmic/nuclear signaling when cells are exposed to injury-producing

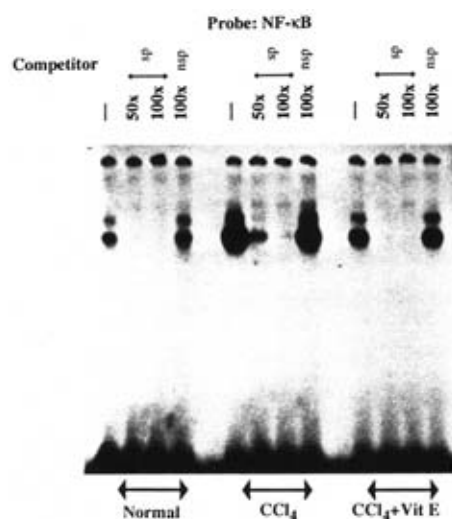


Figure 1. NF-kappa B binding activity assayed by gel retardation using a labelled NF-kappa B site as the DNA probe. Nuclear protein was extracted from the livers of normal mice, mice treated with CCl₄ and mice treated with CCl₄ plus vitamin E. Binding reactions were performed in the absence (–) or presence (+) of nonradioactivity specific (sp) or nonspecific (nsp) competitive oligos at the designated molar excess (from Liu S. L. *et al.* 1995).

conditions. Activators of NF-kappa B include TNF-alpha, IL-1, lipopolysaccharide (LPS), and viral transactivators, such as Tax and HBx (9). NF-kappa B then serves as a second messenger to induce a series of viral and cellular genes in response to an environmental perturbation. Among the genes activated by NF-kappa B are several proinflammatory and cytotoxic cytokines, including colony stimulating factors (GM-CSF and G-CSF), IL-6, IL-2, and TNF-alpha (9). Our work (13-15) and the studies of others (16-18) have shown the association of these cytokines with hepatic injury and fibrogenesis. Particularly germane to this review is the evidence proposed by Baeuerle and co-workers that ROS serve as mediators of NF-kappa B stimulation from such diverse initiators as phorbol 12-myristate 13-acetate (PMA), TNF-alpha, and HBx (19). The most potent *in vitro* inhibitor of NF-kappa B activation described thus far is an antioxidant, pyrrolidine dithiocarbamate (PDTC) (13,19). Moreover, a recent report suggests that glucocorticoids, agents used in the treatment of severe alcoholic hepatitis in man, may act as an immunosuppressant through inhibiting NF-kappa B activation by the induction of I-kappa B-alpha (20).

These reports by others suggest that NF-kappa B may well be a candidate factor that links liver cell injury and oxidative stress with the increased production of cytotoxic cytokines (17). Thus, it appears reasonable to determine the significance of NF-kappa B in the process of hepatic necrosis and fibrogenesis. To our knowledge, prior to our investigations, no previous studies of NF-kappa B activation have been undertaken in hepatic injury systems.

Our studies were among the first to propose a pathophysiologic role for NF-kappa B. Prior to our analysis, the factor had generally been considered to be beneficial to the organism during the injury process.

In our first experiments, acute hepatic injury was induced by injection of CCl₄ in mice, leading to high levels of serum alanine aminotransferase (ALT) and considerable perivenular necrosis. Hepatic injury was almost completely inhibited by intravenous pretreatment of the mice with a water soluble emulsion of vitamin E; i.e. ALT levels fell and liver morphology returned to normal (8). DNA-protein binding to the NF-kappa B consensus sequence, as evaluated by the gel retardation assay, was three-fold higher when nuclear protein was isolated from the livers of CCl₄-treated mice compared to extracts from untreated controls (figure 1). Vitamin E treatment of the mice given the CCl₄ reduced the NF-kappa B binding to levels only slightly above those found in normal mice (figure 1). Treatment of a monocyte/macrophage cell line with CCl₄ led to enhanced NF-kappa B binding and an increase in TNF-alpha messenger RNA levels. TNF-alpha treatment of Hep G2 cells led to enhanced NF-kappa B binding. These data indicate that an acute liver injury model which is caused by free radical induction is associated with enhanced NF-kappa B binding. Abolishing the injury with alpha-tocopherol, a free radical scavenger, also eliminated the increased NF-kappa B binding. It is tempting to speculate that enhanced NF-kappa B expression caused by free radical production/oxidative stress may modulate liver injury, perhaps through an effect on cytotoxic cytokine synthesis. Further investigations of this hypothesis should be undertaken, since recent studies have shown that NF-kappa B may inhibit apoptosis in some circumstances (17, 21).

Growing evidence has also demonstrated that after partial hepatectomy or acute liver injury two transcriptional factors, NF-kappa B and Stat 3, are activated, and that the activation of these factors is probably responsible for enhancing the primary growth response or immediate-early genes, such as *c-fos*, *c-myc*, *c-jun*, *etc.* (21,22). Interestingly, these two factors are commonly activated by cytokines, such as, TNF-alpha, IL-1 and IL-6, which play mediating roles in acute liver damage and regeneration, as well as fibrogenesis. Thus, one can speculate that NF-kappa B may act as a mediator between cytokine release and the occurrence of hepatocellular injury and the onset of hepatocyte regeneration, as well as initiation of hepatic fibrogenesis. The details of this complex cascade have not been elucidated (23).

3. LIPOSOMES AND LIVER DISEASE

Based on the better understanding of mechanisms that initiate liver injury and fibrosis, attempts have been made to improve therapy of hepatocellular injury. Vesicles (liposomes) that are capable of delivering hepatoprotective agents to the liver are an ideal approach to increase local concentration of the agent, to reduce adverse effects, and to

achieve maximal therapeutic efficiency. A great number of chemicals or drugs have been evaluated to reduce hepatocyte damage or necrosis and to inhibit liver fibrogenesis, and some are promising (17). However, none of them is liver-specific or cell type-specific. Thus, developing hepatocyte-specific liposomes that have a high incorporation rate in hepatic parenchymal cells may be potentially useful for reducing hepatocellular damage. Since the majority of liposomes administered intravenously are endocytosed by the reticuloendothelial system (RES), the liposomes are often found to be highly concentrated in Kupffer cells. Hence, it may be possible to improve the efficacy of anti-fibrotic agents by reducing the role of Kupffer cells in the fibrogenic process. When specific antisense oligonucleotides (ONDs) complementary to the 3'-untranslated region of the rat TNF- α mRNA were encapsulated in liposomes and were systematically administered, greater than 65% of the liver-associated ONDs were found in Kupffer cells (24). Moreover, an inhibition of rat TNF- α expression by the ONDs in Kupffer cells was achieved *in vitro* (25). Such a use of liposomes provides for the potential therapeutic benefit of targeting key pathophysiologic pathways in many forms of liver disease.

Liposomes are prepared from a variety of lipids and lipid mixtures, with phospholipids the most commonly used. Generally, two kinds of liposomes (oligolamellar and sterically stabilized small unilamellar liposomes) can be classified according to their composition, chemical features, and pharmacokinetics (26). By the addition of an aqueous medium to a dry phospholipid film, phospholipid bilayers are formed with vortexing, and the bilayers will spontaneously form closed structures with multiple concentric phospholipid envelopes. This type of vesicle is designated as multilamellar or oligolamellar vesicles or liposomes (27). By sonication, extrusion via an extruder or quick freezing and thawing, small unilamellar liposomes can be generated. The oligolamellar liposomes transport substances through membrane-association, whereas small unilamellar liposomes frequently encapsulate substances in the intraliposomal aqueous phase if the substance is water-soluble. Nevertheless, both types of common liposomes are cleared primarily by the RES and only Kupffer cells in the liver have been shown to have high concentrations of their contents. Thus, prolongation of liposome circulating time, as well as reduction of Kupffer cell uptake and enhancement of hepatocyte uptake, are challenges in liposome-targeting research (26-27).

4. PROLONGATION OF LIPOSOME CIRCULATING TIME

Prolongation of liposome circulating time may be attained by avoidance of RES phagocytosis and/or reduction in liposomal aggregation which is attributed to decreased surface hydrophilicity. In addition, liposome clearance is also influenced by its concentration and by plasma protein content. The higher the dose of liposomes, and the less protein bound to liposomes, the longer the circulation half-life was found to be with distearoylphosphatidylcholine (DPC)/cholesterol and egg

phosphatidylcholine (EPC)/dioleoylphosphatidic acid/cholesterol in mice (28). Cisplatin (CDDP) encapsulated in thermosensitive liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) was incorporated with stearylpoloxyethylene (POE) derivatives, which have negative charges. Thus, opsonization of liposomes by other plasma proteins and attachment of liposomes to cell surfaces was attenuated. Accordingly, the Kupffer cell uptake of the liposomes was reduced (29). Injection of clodronate (30) encapsulated in small unilamellar liposomes or dichloromethylene diphosphonate (Cl₂MDP), which was encapsulated in liposomes consisting of DSPC, cholesterol, and stearylamine (SA), affected Kupffer cell function dramatically. After the injection, liver uptake of magnetic particles which were administered intravenously was markedly decreased from 86%, to 40% and 25% at 24 h and 28 h after the injection, respectively, and the particles were redistributed in spleen, lungs and bone marrow (31).

Another alternative to prolong liposome circulating time is to incorporate lecithin-cholesterol liposomes with carboxy group-terminated amphiphilic polymers, such as branched poly(ethylene glycol) (PEG), poly(acryloyl morpholine) and poly(vinylpyrrolidone). Following the biodistribution of liposomes in mice, all three polymers were shown to be effective steric protectors for the liposomes and were able to markedly increase liposome circulating time in a concentration-dependent manner. Unfortunately, the accumulation of these modified liposomes in the liver was decreased (32). Incorporation of PEG conjugates into liposome bilayers has been shown to prolong liposome circulation time in other studies. For example, four hours post-injection of unilamellar PEG liposomes containing monosialoganglioside (GM₁), approximately 55% of the injected dose was still present in blood (33). Amphipathic PEG-phosphatidylethanolamine (PEG-PE) has been shown to prolong the circulating T_{1/2} of the liposomes from 30 minutes to 5 hours (34). Amphipathic PEG not only provides a steric barrier, but also increases the hydrophilicity of the liposome surface, both of which may reduce interaction of the liposome with plasma proteins (including opsonin molecules) and their recognition and uptake by macrophages of the RES (35). It has been shown recently that liver uptake of liposomes is directly related to their surface characteristics. Liver uptake of negatively charged GM₁ inclusion or neutral liposomes does not involve serum components in mice (36). However, rat and human serum significantly enhanced uptake of GM₁-containing liposomes by the perfused liver, probably due to antibody and complement components in rat serum (37).

Size alteration of liposomes may affect their circulating time and only liposomes which are less than 100 nm in size may pass through fenestrae of sinusoidal endothelial cells and gain access to hepatocytes (38). Making liposomes of specific sizes can be undertaken by means of sonication or selection through extrusion with polycarbonate filter membranes with small open meshes.

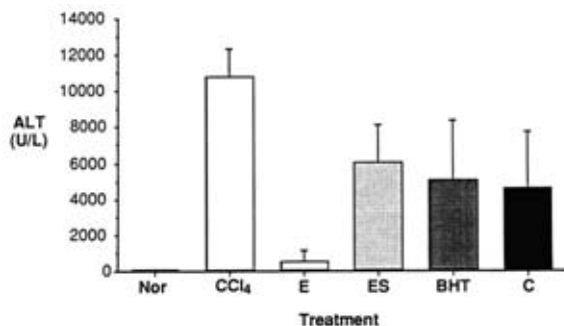


Figure 2. Serum alanine aminotransferase (ALT) activity in mice 24 hours after intraperitoneal administration of CCl₄ (50% CCl₄ 2.5 ml/kg) with or without various pretreatments. The vitamin E (E, 3 mg/mouse), vitamin E succinate (ES, 3 mg/mouse), butylated hydroxytoluene (BHT, 2 mg/mouse) and ascorbic acid 6-palmitate (C, 3 mg/mouse) were entrapped in conventional egg phosphatidylcholine/cholesterol liposomes (size = 100 nm) and injected intravenously into mice 2 hours prior to CCl₄ administration. Nor = normal controls. Each group was composed of at least three mice (from Yao *et al.* 1994).

After extrusion, the size of liposomes is distributed homogeneously. Gel filtration and analytical centrifugation are other methods of selecting homogeneous liposome sizes. Both large ($d > 300$ nm) and small liposomes ($d \approx 75$ nm), made of PEG-PE and cholesterol, were accumulated to elevated levels in spleen and liver. The intermediate sized liposomes ($d = 150$ – 200 nm) were found to have a relatively longer circulation time. Contrary to expectations, the small liposomes labeled with a fluorescent, 1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (DiI), were accumulated in Kupffer cells. There is as yet no evidence available that these small liposomes passed through fenestrated liver endothelium and accumulated in hepatocytes (35). The reason for the reduced circulating time of the small liposomes may be more directly related to the activity of PEG-PE, which may absorb more plasma protein *in vivo* and increase susceptibility to opsonization, thus in turn leading to a more rapid clearance from the circulation.

5. LIPOSOME-TARGETED THERAPY IN LIVER DISEASE

5.1. Attenuation of Drug Side Effects

Utilization of liposomes as a tool of targeting therapy in liver diseases is still in a stage of preclinical evaluation. Most findings are from experiments with animal models and only a few clinical investigations have been reported. Liposome-targeted therapy should theoretically improve treatment outcome as well as reduce adverse effects. With liposome-encapsulated phosphatidyl-dideoxycytidine (DOP-ddC) for treatment of hepatitis B virus, reduction of peripheral neurotoxicity, prolongation of circulating time, and enhancement of hepatic uptake have been demonstrated in mice (39). Marked adverse effects of anti-tumor agents, such as cisplatin, usually impede their applications in patients with malignancies. The toxicity of

liposome-encapsulated cisplatin in rodents was significantly reduced, whereas its therapeutic effects were achieved (40). It was recently reported that local administration of liposomal Adriamycin through the hepatic artery to three patients with metastatic gastric adenocarcinoma significantly lowered common gastrointestinal and myocardial toxicity when compared to patients treated with the free drug. The patients tolerated the treatment with increased doses of Adriamycin (41). A reduction of renal toxicity and an increase in hepatic uptake of cyclosporine (CsA) have been shown for CsA-containing liposomes in a liver transplant model in rats (42). The local concentration of CsA in the liver provided by the liposomes was much higher than by intravenous administration. The immunosuppressive efficacy of the CsA-liposomes was improved with a higher survival rate of transplanted livers. Thus, the encapsulated agent had a longer circulating time, lower plasma concentration, and a similar or better therapeutic efficacy. In addition, it was well tolerated by animals who received the treatment (40). Therefore, liposomal encapsulation, which reduces toxicity of drugs by altering their pharmacokinetics and disposition, may be a potentially effective modality in clinical settings.

5.2. Reduction of Hepatotoxin-Induced Liver Damage

Reduction of hepatotoxin-induced liver injury by liposome-targeted therapy is one possible use of liposomes. Lipid peroxidation is involved in liver injury induced by carbon tetrachloride (CCl₄) and other hepatotoxins. Intravenous injection of vitamin E (VE)-containing liposomes has been shown to be highly effective in the treatment of the damage, probably by means of the antioxidant and radical scavenging action of VE. This improvement in hepatocellular necrosis occurred despite the majority of the liposomes being entrapped in Kupffer cells (43). Conventional egg phosphatidylcholine/cholesterol (EPC-Ch) liposomes containing alpha-tocopherol were generated at an approximate size of 100 nm. These VE-containing liposomes (3 mg/mouse) were intravenously injected into mice two hours prior to subsequent CCl₄ challenge. The data shown in (figure 2) demonstrate that VE-containing liposomes gave rise to better protection from hepatocellular injury than did VE succinate, butylated hydroxytoluene (BHT), or ascorbic acid 6-palmitate entrapped in the same type of liposomes. Improvement of the CCl₄-induced acute liver injury was indicated by decreased serum ALT activity and improved liver histology. Liposomal VE also markedly reduced lethal dose CCl₄-induced mortality in mice (43). We recently labeled the conventional liposomes with asialofetuin (AF), a ligand for asialoglycoprotein receptors in hepatocytes. The AF-labeling not only markedly increased the liver up-take of the liposomes, but also significantly improved efficiency of drug delivery to the liver. VE-containing AF-liposomes at a dose of 1 mg/mouse of VE achieved similar protection compared to VE-liposomes at 3 mg/mouse on CCl₄-induced acute liver injury (44).

In addition, the metabolism of the liposomes in rat liver did not lead to an increase in products of lipid peroxidation (45). Numerous drugs which display toxicity

to the liver, such as acetaminophen, may elicit lipid peroxidation. Metabolism of ethanol may also evoke oxidant insult to the liver. Thus, liposome-targeted anti-oxidant treatments, e.g., VE and its analogs (Trolox C, TGPS) (46), or superoxide dismutase (SOD) (45), may benefit patients with drug-associated liver damage or alcoholic liver disease. Anti-oxidant liposome formulations may also improve the preservation of donor livers during the process of liver transplantation, because ischemia-reperfusion during transplantation induces an oxidant injury to the sinusoidal endothelial and parenchymal cells (17).

5.3. Cationic Liposomes for Gene Delivery

A variety of strategies and methods for hepatic gene transfer have been evaluated (47-50). Cationic liposomes have been used for gene transfer to a variety of cell types or for somatic gene therapy. Cationic liposomes can be formed with positively charged lipids, such as N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium (DOTMA) with a neutral lipid, dioleoylphosphatidylethanolamine (DOPE). The resulting liposomes (Lipofectin) then spontaneously interact with cell surfaces which contain negative charges, and deliver associated polynucleotide complexes to the cell interior. One characteristic of cationic liposomes is that they may avoid degradation of entrapped DNA in lysosomal compartments, in which conventional liposomes and their entrapped contents are readily degraded (51). DOTMA spontaneously interacts with DNA or RNA, resulting in a liposome/polynucleotide complex that captures essentially 100% of the polynucleotide for a high efficiency of gene delivery (52). For *in vivo* gene transfer, another novel cationic lipid, dimyristoyloxypropyl-3-dimethylhydroxyethyl ammonium (DMRIE), has been employed in DNA-liposome complexes with DOPE. This substitution apparently both improves transfection efficiency and allows increased amounts of DNA to be delivered *in vivo*. DMRIE-DOPE liposomes have been shown to be well-tolerated *in vivo* and to be highly efficacious in gene transfer (53). Liposome-DNA plasmid complexes display much better systemic gene delivery efficiency than direct intravenous injection of DNA plasmids since the plasmids encapsulated in liposomes were effectively protected from environmental degradation (54). Similar to conventional liposomes, cationic liposome-plasmid DNA complexes were quickly cleared from plasma when administered intravenously, and 50-60% of the injected dose was taken up by the liver within few minutes after administration if the liposome size was large (600-1200 nm). Again, Kupffer cells were the major cell type to take up the liposomes through phagocytosis (55). When pSV2CAT, a chloramphenicol acetyltransferase (CAT) reporter gene plasmid was associated with asialofetuin-labelled liposomes, CAT activity in the liver was significantly higher than unlabeled liposomes when administered into the portal vein of an adult mouse. Immunohistochemical staining showed that CAT was localized in parenchymal cells in the periportal areas (56).

Lipofectin displays low organ- or tissue-specificity. Thus, administration of the liposomes through the portal vein may achieve better transfection in the liver

than intravenous injection. An attempt has been made to transfer the hepatitis C virus (HCV) genome into rat liver with Lipofectin, and the expression of HCV RNA transcripts and HCV core protein has been confirmed (57). Because there are asialoglycoprotein receptors on hepatocytes and Hep G2 cells, AGP-labeled cationic liposomes have been shown to be highly effective in targeting Hep G2 cells through a receptor-mediated gene transfer mechanism (58). Recently, it was reported that a polycationic amphipathic molecule, lipospermine (Transfectam) was a particularly efficient and specific transfection agent for Hep G2 cells when galactose ligands were added to the system (59-60). The presence of a thiol-reactive phospholipid derivative, N-4-(p-maleimidophenyl)butyryl dipalmitoylphosphatidylethanolamine (MPB-DPPE), in electrically neutral lipospermine/DNA particles, resulted in more than an 100-fold increased transfection efficiency of Hep G2 cells and 3T3 fibroblasts in comparison to particles without MPB-DPPE. It was assumed that such particles react with thiol groups present on the surface of the cells, leading to their covalent anchoring, a process that is probably followed by endocytosis of the complex (61).

Cationic liposomes appear to be potentially useful carriers for *in vivo* gene transfer and there is a catalogue of hepatic diseases or disorders that might lend themselves to gene therapy via liposome targeting; the list includes viral hepatitides, metabolic disorders such as alpha-1-antitrypsin (AAT) deficiency, and hepatic malignancies. Features of cationic liposomes allow for a high entrapment rate of polynucleotides and efficient transfer, which may improve the expression of transferred genes (49). For example, a plasmid which contained human AAT gene sequences and was entrapped in small liposomes composed of EPC, brain phosphatidylserine and cholesterol, has been transferred into mouse hepatocytes, and the expression of human AAT was identified in the liver of the transgenic mice (62). Other inherited disorders, such as hemophilia and low density lipoprotein (LDL) receptor deficiency can be treated by transferring replacement genes to the liver using liposome-plasmid DNA complexes (63).

Antisense can specifically inhibit mRNA translation by complementary coupling to targeted mRNA sequences (64). A major barrier for the development of antisense oligodeoxynucleotides (ODNs) is the permeability of ODNs to the plasma membrane. ODNs must be present at sufficiently high concentration in the cell for RNA binding to occur. It is common that when ODNs are added to cell culture medium, they are not efficiently transported into the cytoplasm or nucleus of most cells, resulting in a poor or non-existent antisense effect (65). Since cationic liposomes are capable of delivering DNA to the cells and potentiating its movement through the plasma membrane, Lipofectin has been tested for transferring ODNs into cells, leading to antisense inhibition of gene expression. However, Lipofectin is serum sensitive and can not be used in cultures in which serum is required for cell growth or survival. A new cationic lipid, GS2888, has been synthesized and coupled with DOPE (Cytofectin), and its

efficient delivery of ODNs has been evaluated in variety of cell types (66). It has been shown that GS2888/Cytofectin could efficiently transfer ODNs or plasmid DNA into different cell types in the presence of serum, and the transferring efficiency was up to 20-fold higher than with Lipofectin. An inhibition of gene expression could be achieved with nanomolar concentration of ODNs with GS2888/Cytofectin. Thus, this type of cationic liposome appears to be useful for delivering ODNs and for inhibiting expression of important genes *in vitro*. Specific antisense ODNs complementary to duck hepatitis B virus were encapsulated in cationic liposomes, and the entrapped ODNs were more stable in serum and were shown to have better targeting effects to the liver. The systematically administrated liposomal ODNs markedly inhibited replication of duck HBV in the liver and lowered serum viral proteins five days after their administration. However, higher doses of random ODNs also displayed a significant inhibition of duck HBV (67).

6. CONCLUSION

For investigators of other organ systems, the fact that the vast majority of systematically administered liposomes are taken up by the liver is a serious problem in designing therapeutics. For those involved in the study of liver disease, the natural targeting of liposomes is of great potential value. Liposomes can be readily employed to decrease toxicity of therapeutic agents to other organs, while at the same time enhancing the drug concentration that affects the liver. Moreover, liposomes offer opportunities for the targeting of nucleotides to the liver in attempts to develop new approaches for the gene therapy of a series of liver diseases. A great challenge faces the investigator who wishes to target liposomes to hepatocytes or Kupffer cells *in vivo*; however the possible benefits from the use of these valuable vesicles are indeed substantial.

7. ACKNOWLEDGEMENT

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