PHARMACEUTICAL NANOTECHNOLOGY

Liposomes Incorporating a *Plasmodium* Amino Acid Sequence Target Heparan Sulfate Binding Sites in Liver

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ABSTRACT: Previous studies demonstrated that intravenously administered liposomes, incorporating a peptide from the *Plasmodium* circumsporozoite protein, accumulate rapidly and selectively in mouse liver. The present investigation was designed to determine the molecular components in liver responsible for liposome targeting. Studies of liver tissue slices demonstrated that immunoreactivity for heparan sulfate proteoglycan (HSPG), but not other tested proteoglycans, was distributed along sinusoidal borders of liver; this immunoreactivity appeared associated with nonparenchymal cells of the sinusoids and with the basolateral portion of hepatocytes. Peptide-containing liposomes bound to liver tissue in a pattern similar to the distribution of heparan sulfate immunoreactivity, either after intravenous injection of liposomes *in vivo* or after incubation of liposomes with liver slices *in vitro*. Control liposomes, without the peptide, displayed very light binding without a pattern. Pretreatment of liver slices with heparinase, but not chondroitinase or hyaluronidase, eliminated peptide-containing liposome binding, but did not affect binding of control liposomes. Coincubation of peptide-containing liposomes with heparin, but not with other glycosaminoglycans, markedly inhibited liposome binding to liver slices. *N*-desulfated and *O*-desulfated heparins individually were less effective inhibitors of liposome binding than was heparin. These results indicate that liposomes containing a peptide from *Plasmodium* target liver tissue by binding to HSPGs in the extracellular matrix. J Pharm Sci 97:3257–3273, 2008

Keywords: heparan sulfate; hepatocytes; imaging methods; liposome; liver slice; nanotechnology; peptide delivery; *Plasmodium*; targeted drug delivery.

INTRODUCTION

Understanding mechanisms that mediate the targeting of therapeutic nanoparticles to specific organs or tissues *in vivo* remains an important goal of efforts to develop drug delivery systems. Success in achieving this goal will be dependent upon appropriate selections, both of a model organ system and also of a targeting device. The liver presents an attractive model organ for studies of nanoparticle targeting and uptake, because

Abbreviations: chon-ase, chondroitinase; chon-S, chondroitin sulfate; CSP, circumsporozoite protein; cv, central vein; hep-S, heparan sulfate; hep-ase, heparanase; HSPG, heparan sulfate proteoglycan; hyal-ase, hyaluronidase; ICC, immunocytochemistry; lipos, liposomes.

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the combination of relatively large fenestrations of the endothelial lining and the absence of a significant basement membrane between endothelial cells and hepatic parenchyma allow for less impeded particle movement from circulating blood to parenchymal cells.[1–3]

In the search for a targeting device, recently, we[4] demonstrated remarkably specific targeting to liver tissue following intravenous injections of liposomes that incorporated a peptide derived from *Plasmodium*, the infectious organism that leads to malaria.[5–8] Numerous microorganisms have developed highly effective organ specific targeting strategies for host invasion.[9–13] It is our view that engineering these targeting strategies into nanoparticle delivery system offers the best chance of success for the development of highly specific targeting systems.

The targeting peptide chosen for these studies included the conserved region I from the circumsporozoite protein (CSP) of *Plasmodium* and a consensus HSPG binding sequence.[9–13] These peptides are referred to in the literature as "region I plus".[13] In our studies, the region I plus peptide was attached to a lipid-polyethyleneglycol bioconjugate and incorporated into fluorescently labeled liposomes.

The remarkable targeting specificity of these peptide-containing liposomes underscores the importance of identifying the structural and molecular characteristics of the liver that serve to attract or bind the liposomes.[14–20] Recent work by Ancsin and Kisilevsky[13] has shown that a similar *Plasmodium* region I plus peptide binds strongly to affinity columns of heparin and heparan sulfate. Their finding suggests that this amino acid region is at least partially responsible for *Plasmodium* targeting to liver *in vivo*, and functions by recognition of highly-sulfated HSPG's that are characteristic of extracellular matrix associated with liver sinusoidal borders.[8,13,16,21,22] Proteoglycans normally serve a variety of cellular and physiologic functions, through interactions with their respective ligands.[23–26] Moreover, adherence to proteoglycans has been exploited by a variety of microorganisms as an initial step of host invasion,[12] as is exemplified by *Plasmodium* sporozoite targeting of liver.[5–10,12,16,27]

The purpose of the present investigation was to characterize the extracellular sites and molecular components that bind the *Plasmodium* peptide-containing liposomes[4] in liver. First, immunocytochemical studies revealed distributions within the liver of different classes of proteoglycans; heparan sulfate proteoglycan (HSPG) immunoreactivity in particular was distributed along sinusoidal borders in a pattern that mimics the distribution of peptide-containing liposomes after intravenous administration. Second, treatment of the liver slices with enzymes that degrade specific proteoglycans demonstrated that exposure of liver slices to heparinase disrupted liposome binding. Third, competitive inhibition experiments demonstrated that exposing the peptide-containing liposomes to soluble heparin interfered with liposome binding to slices of liver. Taken together, these results indicate that HSPGs in the liver serve as a binding site for peptide-containing liposomes injected systemically in experimental animals.

EXPERIMENTAL METHODS

Peptide Synthesis

The peptide acetyl-CKNEKKNKIERNNKLKQPP-amide consisted of an *N*-terminal acetylcysteine followed by amino acids 76–93 of the *N*-terminal region of the CSP of *Plasmodium berghei*, Anka strain (Swiss Protein entry name: CSP_PLABA; Primary accession number: P23093). This peptide was synthesized by Fmoc chemistry using a FastMoc™ protocol.[28] Cleavage from the resin and purification were performed as described previously.[29,30] Mass spectrometry was performed using alpha matrix[31] in an ABI Voyager DE-STR MALDI-TOF instrument operating in reflectance mode, with positive polarity, delayed extraction, and an acquisition range of 1100–3000 Da. The mass spectrum exhibited a single molecular species with the correct mass + 1 of 2351 Da (100% carbon-12) followed by additional intensities at 2352–2355 corresponding to the expected binomial distribution of 1, 2, 3, and 4 atoms of carbon-13.

Lipid Synthesis

1,2-Dierucoyl-*sn*-3-phosphatidylcholine (di22:1-PC) was obtained from Avanti Polar Lipids (Alabaster, AL). 1,2-Dierucoyl-*sn*-3-phosphatidylethanolamine (di22:1-PE) was synthesized by base-exchange of di22:1-PC with ethanolamine, catalyzed by *Streptomyces* sp. phospholipase D (Sigma, St. Louis, MO).

The synthesis of 1,2-dierucoyl-*sn*-3-aminopropane (di22:1-AP) was carried out in three steps. First, *sn*-3-aminopropanediol (Aldrich, Milwaukee, WI) was converted to *sn*-3-(t-BOC)aminopropane-