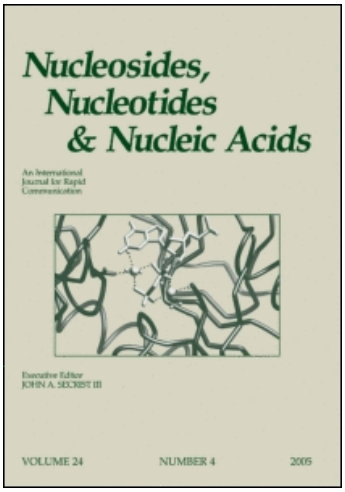

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Lipophilic Derivatization of the Antiviral Drug 9-(2-Phosphonylmethoxyethyl)adenine and Its Incorporation into a Lactosylated Lipid Carrier to Improve Its Liver Uptake

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LIPOPHILIC DERIVATIZATION OF THE ANTI-VIRAL DRUG 9-(2-PHOSPHONYLMETHOXYETHYL)ADENINE AND ITS INCORPORATION INTO A LACTOSYLATED LIPID CARRIER TO IMPROVE ITS LIVER UPTAKE

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ABSTRACT: Lipophilic derivatization of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) with lithocholic acid-3-oleate and its subsequent incorporation into a lactosylated lipid carrier was found to substantially increase uptake of the drug by the liver. Competition experiments with asialofetuin point to a major role of the parenchymal liver cell, the main site of hepatitis B virus infection.

Worldwide, more than 350 million people suffer from chronic hepatitis B. This liver disease is caused by an infection of the parenchymal liver cells with the hepatitis B virus (HBV) and is associated with a highly increased incidence of cirrhosis and hepatocellulair carcinoma[1,2]. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA), an acyclic nucleoside phosphonate analog has been shown to inhibit the replication of HBV in cultured cells[3,4]. Unfortunately, only a limited amount of PMEA is taken up by parenchymal liver cells upon intravenous injection. Most of the administered PMEA is rapidly cleared from the circulation by the kidneys and excreted in the urine[5]. However, a substantial amount of the administered

dose of acyclic nucleoside phosphonate analogs is retained in the kidneys, causing significant nephrotoxicity[6,7]. Selective delivery of PMEA to parenchymal liver cells would therefore improve its therapeutic efficacy and reduce the toxic side-effects.

In this study we investigated the possible use of lactosylated neo high-density lipoprotein (NeoHDL) as a carrier for PMEA. Lactosylated NeoHDL is a synthetic particle, consisting of a lipid moiety and lactosylated apoproteins. This lipid carrier is specifically internalized via the asialoglycoprotein receptor on parenchymal liver cells, and transported to the lysosomes[8,9]. A lipophilic prodrug of PMEA was synthesized to allow incorporation into the lipid moiety of the carrier.

To be able to monitor the biological fate of the drug, [^{3}H]PMEA was used. [^{3}H]PMEA was derivatized with lithocholate-3-oleate in a two-step reaction (FIG. 1). First, [^{3}H]PMEA was derivatized with ethylenediamine, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as coupling reagent.
The product, [^{3}H]PMEA-NH_2, contains a phosphonoamidate bond, which is stable at neutral pH, but is hydrolyzed in the acidic environment of the lysosomes. The drug will be released in its pharmacologically active form at the lysosomal pH. Next, [^{3}H]PMEA-NH_2 was conjugated with the pentafluorphenyl ester of lithocholic acid-3-oleate (LO-PFP), resulting in [^{3}H]PMEA-NH-lithocholate-3-oleate ([^{3}H]PMEA-LO). The lipophilic prodrug was incorporated into neoHDL using the same procedure as was described earlier for 3',5'-dioleoyl-5-iodo-2'-[6-^{3}H]deoxyuridine.[8,9] In brief, [^{3}H]PMEA-LO was cosonicated with lipids and apoprotein A-I. After sonication, the resulting [^{3}H]PMEA-LO-loaded emulsion was subjected to a density gradient ultracentrifugation. Particles in the density range of native HDL (1.08-1.18 g/ml) were selected and purified by gelfiltration chromatography. Finally, [^{3}H]PMEA-LO-loaded NeoHDL was lactosylated by means of reductive lactosamination.

The resulting lactosylated [^{3}H]PMEA-LO-loaded NeoHDL particles were intravenously injected into male wistar rats. The particles were cleared from plasma with a half-life of less than 4 min (FIG. 2).At 20 min after injection, the liver contained more than 60% of the injected dose, whereas kidney uptake was less than 2% (free [^{3}H]PMEA: liver and kidney uptake, 5% and 40%, respectively).

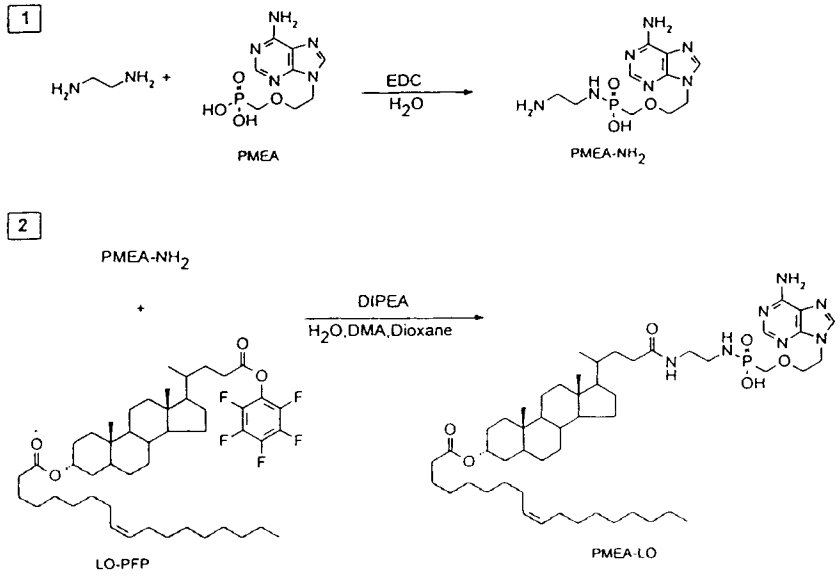

FIG. 1: Lipophilic derivatization of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) with the pentafluorphenyl ester of lithocholic acid-3-oleate (LO-PFP), using ethylene diamine as acid-labile spacer.

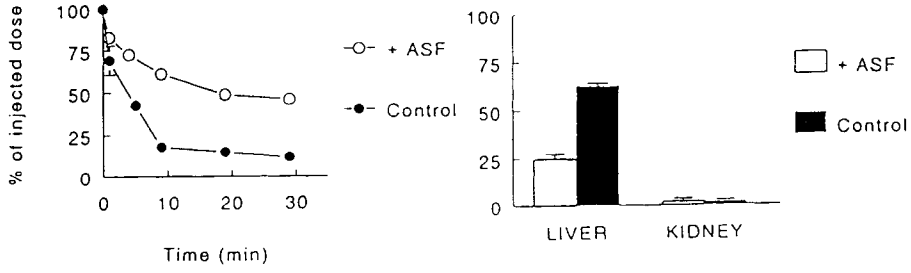

FIG. 2: Plasma clearance and association to the liver and kidney of lactosylated [^{3}H]PMEA-LO-loaded NeoHDL in (asialofetuin pretreated) rats. Radioactivity in the liver and kidney was determined at 20 and 30 minutes after injection, respectively. Values are means ± S.E.M. of 2 rats.

Preinjection with asialofetuin, a substrate which specifically inhibits ligand uptake by the asialoglycoprotein receptor on parenchymal liver cells, reduced the liver uptake of lactosylated [^{3}H]PMEA-LO-loaded NeoHDL by 60%, indicating that this receptor is involved in the uptake (FIG. 2).

In conclusion, the lipophilic derivatization of PMEA and its subsequent incorporation into a lactosylated lipid carrier results in an increased liver uptake, probably by the liver parenchymal cells.This increase may result in an improved therapeutic efficacy against HBV.

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