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Uptake of phosphatidylserine-containing liposomes by liver sinusoidal endothelial cells in the serum-free perfused rat liver

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Abstract

We studied the kinetics of hepatic uptake of liposomes during serum-free recirculating perfusion of rat livers. Liposomes consisted of phosphatidylcholine, cholesterol and phosphatidylserine in a 6:4:0 or a 3:4:3 molar ratio and were radiolabelled with [^{3}H]cholesteryl oleyl ether. The negatively charged liposomes were taken up to a 10-fold higher extent than the neutral ones. Hepatic uptake of fluorescently labelled liposomes was examined by fluorescence microscopy. The neutral liposomes displayed a typical Kupffer cell distribution pattern, in addition to weak diffuse staining of the parenchyma, while the negatively charged liposomes showed a characteristic sinusoidal lining pattern, consistent with an endothelial localization. In addition, scattered Kupffer cell staining was distinguished as well as diffuse parenchymal fluorescence. The mainly endothelial localisation of the negatively charged liposomes was confirmed by determining radioactivity in endothelial and Kupffer cells isolated following a 1-h perfusion. Perfusion in the presence of polyinosinic acid, an inhibitor of scavenger receptor activity, reduced the rate of uptake of the negatively charged liposomes twofold, indicating the involvement of this receptor in the elimination mechanism. These results are compatible with earlier in vitro studies on liposome uptake by isolated endothelial cells and Kupffer cells, which showed that in the absence of serum also endothelial cells in situ are able to take up massive amounts of negatively charged liposomes. The present results emphasize that the high in vitro endothelial cell uptake in the absence of serum from earlier observations was not an artifact induced by the cell isolation procedure.

Keywords: Phosphatidylserine; Liposome; Liver

1. Introduction

The mechanisms involved in the elimination of liposomes from the blood compartment are still not fully understood. Upon parenteral administration, liposomes are taken up by macrophages of the spleen and the liver and, depending on liposome size and composition, hepatocytes may also significantly contribute to their uptake from the blood [1]. An important role in these uptake mechanisms has been attributed to serum proteins that, in relation to macrophage uptake, are often referred to as opsonins [2]. The involvement of serum or serum proteins in the clearance of liposomes from the blood appears to be very complex. Liposomal parameters such as lipid composition and charge are determining factors in the process of blood clearance, while also considerable species differences have been reported [3, 4]. In addition to serum-dependent liposome uptake mechanisms, serum-independent liposome uptake has been reported in a liver perfusion system in mice [5, 6]. In rats liposomes containing phosphatidylserine (PS) are efficiently eliminated from the blood by cells of the mononuclear phagocyte system, predominantly Kupffer cells in the liver. Hepatocytes have also been

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shown to contribute to the uptake of small liposomes containing PS, but we have consistently found liver endothelial cells not to participate in the elimination of liposomes without ligands [7, 8]. By contrast, in primary cultures of liver endothelial cells, we demonstrated that binding and uptake by these cells of liposomes containing PS were at least as efficient as that by Kupffer cells [9]. This in vitro association of PS-containing liposomes with liver endothelial cells was attributed to scavenger receptor activity. Depending on the PS content, the interaction of liposomes with scavenger receptors was partly or even completely inhibited by serum. The latter observation may explain at least partly the in vitro–in vivo differences in the capability of liver endothelial cells to take up liposomes containing PS. However, besides the continuous presence of serum proteins in vivo, another important difference with the in vitro situation is the obvious fact that, in vivo, liver endothelial cells co-exist in close proximity to or even in contact with Kupffer cells and hepatocytes, which may affect their functions. To gain more insight in the mechanisms underlying liposomal blood clearance by liver cells in the intact liver, we applied a controlled, isolated rat liver circulating perfusion system [10, 11].

In this study, we investigated the interaction of radioactively or fluorescently labelled negatively charged liposomes containing 30% PS and uncharged neutral liposomes in the intact perfused rat liver. In this way, hepatic liposome elimination can be studied in the absence of blood constituents but in an otherwise intact physiological environment. Liver uptake, uptake rate and intrahepatic distribution of the liposomes were determined. The possible involvement of scavenger receptors in liposome uptake in the intact perfused liver was assessed during perfusions in the presence of polyinosinic acid, an established inhibitor of scavenger receptor-mediated uptake.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (PC) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (Chol) was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland). Soybean phosphatidylserine (PS) was supplied by the American Lecithin Company (Oxford, Connecticut, USA). [1α, 2α(*n*)-^{3}H] Cholesteryl oleyl ether (^{3}H-COE) was obtained from Amersham Pharmacia biotech (Freiburg, Germany). DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and polyinosinic acid (Poly(I))-potassium salt were obtained from Sigma-Aldrich Co., USA. All other chemicals obtained from commercial sources were analytical grade or the best grade available.

2.2. Animals

Specified pathogen-free (SPF) male Wag/Rij rats (Harlan, Horst, The Netherlands) or Wistar rats (Boehringer Ingelheim, Germany) were kept under standard animal laboratory conditions and had free access to standard lab chow and water. The experimental protocols were approved by the local committee for care and use of laboratory animals.

2.3. Liposome preparation

The liposomes used in this study were composed of PC, Chol and PS in molar ratios of 3:4:3 (30% PS) or 6:4:0 (0% PS), respectively. The liposomes contained a tracer amount of the radioactive label [1α, 2α(*n*)-^{3}H] Cholesteryl oleyl ether. In some experiments 0.25 mol% of the fluoresecent marker 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was incorporated in the liposomal bilayers. Liposomes were produced by a conventional rotary evaporation method [12] and sized by extrusion through polycarbonate filters with a pore size of 200 nm (Costar Nuclepore, Tübingen, Germany) using a commercially available device (EmulsiFlex-C5, Avestin Inc., Ottawa, Ontario, Canada). PC content was measured by a phospholipase D/cholinoxidase/PAP-test (Phospholipids B, WAKO Chemicals GmbH, Neuss, Germany) and the liposome concentration was adjusted to 10 mg of total lipid/ml using Krebs–Henseleit buffer (118 mM NaCl, 4.74 mM KCl, 0.59 mM KH_2PO_4, 0.59 mM Na_2HPO_4, 24.90 mM $NaHCO_3$, and 5.50 mM D-glucose) without $MgCl_2$ and $CaCl_2$. Liposome size was determined by dynamic light scattering either using a Zetasizer 4 or a Zetasizer 3000HS (Malvern GmbH, Herrenberg, Germany). The diameter of the liposome preparations used was 200±38 nm with a polydispersity coefficient of not larger than 0.2. ζ-potential of the liposomes was determined using a NICOMP model 380 ZLS zeta potential/particle sizer (NICOMP particle sizing systems, Santa Barbara, CA, USA). The ζ-potentials of 0% PS and 30% PS liposomes were −4.6 and −27.3 mV, respectively. Prior to the perfusions, the liposomes were diluted to a concentration of 0.4 mg/ml with Krebs–Henseleit buffer, the medium was warmed to 37 °C and the pH was adjusted to 7.4. Addition of 1.25 mM $CaCl_2$ and 1.19 mM $MgCl_2$ (final concentrations) was done immediately before the perfusion.

2.4. Oxygenation of the perfusion medium

For the oxygenation of the perfusion medium, Krebs–Henseleit buffer (washing buffer) or liposomal preparation was passed through a highly efficient homemade oxygenator, constructed from an HPLC inline degasser cassette (1 channel chamber, OmniLab AG, Mettmenstetten, Switzerland) using the vacuum connection as a carbogen (95% O_2, 5% CO_2) inlet. The perfusion medium was pumped