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In Vitro Transfer of Phosphatidylcholines and Their Ether Analogs by a Human and Rat Plasma Exchange Factor

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The phospholipid transfer properties of human and rat plasma have been studied using radiolabeled phosphatidylcholines (PCs) and diether PCs as well as a series of fluorescent PCs. The PC transfer activities of human and rat lipoprotein deficient sera are similar. Lipoprotein deficient rat serum transfers PC at a rate that is similar, if not identical, to the rate of transfer of the ether analogs of PC. These results suggest that PC ethers might be used to identify the nonhydrolytic metabolic routes of PCs.

In plasma the phosphatidylcholines (PCs) circulate as components of lipoproteins (1). There are numerous routes that can contribute to the turnover of plasma PC; one of these involves receptor mediated endocytosis or fluid phase pinocytosis of the entire lipoprotein(2); turnover times of whole lipoproteins have been determined in both rat and man using suitable radiolabeled markers(3). Another route involves spontaneous transfer of a PC from a lipoprotein to cells in a process that involves movement of the PC monomer through the intervening aqueous phase that separates the cell and lipoprotein surfaces. This rate can be predicted on the basis of in vitro transfer rates because the rate-limiting step is desorption from the lipoprotein surface(4). More importantly the spontaneous transfer times of the physiologically important PCs occur on a time scale that is far too slow to compete effectively with other processes(5). A third putative route is the transfer of PC from lipoproteins to cells via transfer factors that have been reported by numerous investigators(6-9). However, the contribution of this process to the plasma PC turnover is complicated by the action of three important plasma enzymes that hydrolyze PC. These are lecithin:cholesterol acyltransferase (LCAT) and hepatic (HL) and lipoprotein lipases (LPL) (1).

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We have recently described the use of diether analogs of LCAT (10); PC ethers are competitive inhibitors of LCAT, the activity of which is reduced by the fraction of the total PC that is composed of PC ether; i.e., $K_i = K_m$. Thus, if PC ethers are mobilized by transfer factors in a manner that is similar to that of natural PCs, they could be used in vivo to estimate the contribution of the transfer factors to the nonhydrolytic turnover of PC. Herein we compare the transfer rates of PC, their diether analogs using human and rat lipoprotein deficient serum (LPDS) as a source of transfer activity.

EXPERIMENTAL:

ApoA-I was obtained by a published procedure (11). The pyrene labeled lipids were synthesized according to Massey et al.(4). Distearyl (DSPC) and dipalmityl (DPPC) ethers were purchased from Bachem. Dimyristyl PC (DMPC) ether was prepared according to Massey et al. (12) 1-Palmitoyl-2-oleoyl PC (POPC) was from Avanti. POPC ether was synthesized according to Newaz and Pownall (manuscript in preparation). Radiolabeled lipids were prepared by the demethylation-methylation method of Stoffel(13) and Patel (14). All PCs and PC ethers are, respectively, sn-1,2-diacyl and sn-1,2-dialkoxy glycero-sn-3-phosphocholine derivatives.

Preparation of Lipoprotein Deficient Serum: A partially purified transfer factor from human and rat plasma was obtained by adjusting 30 ml plasma to 3M NaCl and passing it through a 60 ml column of Phenyl Sepharose. Following a 200 ml wash with 3 M NaCl the exchange activity was eluted with pure water. This preparation was used to compare the total exchange activities of human and rat plasma in a buffer of 100 mM NaCl, 0.01% EDTA, 0.01% Azide, and 10 mM Tris, pH 7.4.

Transfer Assay: Two assays of exchange activity were used. One was a fluorescent assay described by Massey et al. (5) in which the rate of disappearance of pyrene excimer fluorescence was monitored. Briefly, reassembled high density lipoproteins (R-HDL), prepared by the method of Matz and Jonas (15), and composed of apoA-I, POPC, and a pyrene labeled PC (1:98:2), were mixed with a 10 fold excess of unlabeled R-HDL. After addition of exchange factor the rate of disappearance was recorded. The rates were corrected for the spontaneous transfer rates (4). Rate constants were obtained by a least squares treatment of the data.

In the second assay, which was used for radiolabeled PCs and PC ethers, the rate was determined by measuring the transfer of the radiolabeled lipid from R-HDL to hand shaken liposomes of a brominated egg PC as a function of time according to Dawidowicz and Rothman (16). The liposomes were pelleted by low speed centrifugation and the radioactivity determined by liquid scintillation counting.

RESULTS:

The PC transfer activities of human and rat plasma tested by the pyrene excimer method using labeled R-HDL as the donor and excess unlabeled R-HDL as the acceptor. The labels were sn-1-acyl-sn-2-[9-(3-pyrenyl)nonanoyl]-sn-3-phosphocholines; the acyl groups at the sn-1-position were myristoyl (MPNPC), palmitoyl (PPNPC), stearoyl(SPNPC), and oleoyl (OPNPC). The halftimes for

Table 1

Comparison of RAT and Plasma Exchange Protein Activities

Label[a]	μmoles lipid transferred/hr /mg exchange protein[b]	Spontaneous Transfer Halftime[c] Rate, Min	Source of Activity
MPNPC	3.8±0.1	8	rat plasma
PPNPC	6.8±3.2	55	rat plasma
OPNPC	4.2±0.9	72	rat plasma
SPNPC	2.7±0.3	270	rat plasma
SPNPC	3.0±0.2	270	human plasma

[a]Donor=fluorescent labeled PC:POPC:apoA-I (2:98:1); 0.063 μmol PC/assay.
Acceptor= POPC single bilayer vesicles formed by sonication; 1.6 μmol/assay.
[b]Accelerated - spontaneous rates
[c]From reference 5.

transfer are given in Table 1 along with the spontaneous rates according to Massey et al. (5). These data show that there is little difference between the rates of transfer of the different PCs by rat LPDS even though the rates of spontaneous transfer decrease rapidly as a function of increasing chain length (5). The differences in the rates of spontaneous and LPDS facilitated transfer increase as a function of the chainlength of the PC and for the long-chained species the protein-mediated pathway is the dominant route. In separate experiments we verified that increasing the amount of transfer factor five times increased the rate by five times, thereby demonstrating that the transfer is linearly dose-dependent. In one case we compared the activities of human and rat LPDS and observed nearly identical rates (Table 1). These rates were not effected by heat inactivation of LPDS (50°, 1 hr).

We also compared the transfer rates of a series of diacyl PCs with their ether analogs using the radiolabeled assay. We observed only minor differences in their LPDS mediated transfer rates (Figure 1, Table 2). The major difference was the slightly faster rate of POPC transfer relative to its ether analog. In the case of DPPC and DSPC and their ether analogs, very similar values were observed. The rate of spontaneous transfer of DMPC was too fast to measure by this assay.

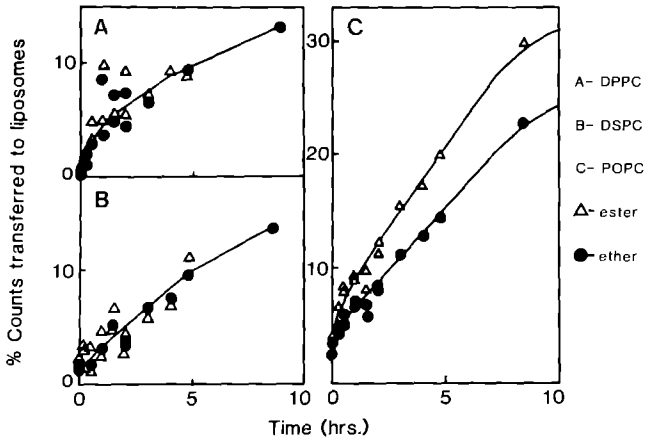

Figure 1: In vitro transfer of [^{14}C]-PC and [^{3}H]-PC diethers from reassembled HDL ([^{3}H]-diether PC:[^{14}C]-diester PC: POPC:apoA-I) (5:5:90:1) (M:M:M:M) to brominated egg PC liposomes by a rat plasma exchange protein. Each assay contained 1 μ mole donor phospholipid, 15 μmole acceptor phospholipid and 1 mg exchange protein or buffer (control) in 250 μl. Samples were incubated at 37°C and exchange activity was stopped by addition of 100 μl of 10 mg/ml $HgCl_2$. 3 ml of 0.1 M Tris, pH 7.4 buffer was added and the sample spun at 11,000 g for 30 mins. The supernatant was decanted and the pellet suspended in 6 ml scintillation fluid. In each case the passive exchange rate (no protein) was subtracted from the accelerated rate.

DISCUSSION:

We have verified the observation that both human and rat LPDS contain a PC transfer factor. We have also shown that the same amount of transfer activity is contained in a given amount of rat and human LPDS. Most importantly, we have shown that ether analogs of diacyl PCs are transferred as efficiently as the PCs are. By using heat inactivated serum we ensured that

Table 2
Rat LPDS Mediated Transfer of PC and PC Ethers

	% PC Transferred/5 hr
DMPC	9
DMPC ether	9
DPPC	10
DPPC ether	10
POPC	20
POPC ether	15

none of the transfer was due to the easily transferable products of HL, LPL, or LCAT, lysoPC. These data suggest that *in vivo* the transfer protein represents one exchange route by which PCs might be removed from the plasma compartment. These results also suggest that the PC ethers, which are not natural components of mammalian plasma, and which are not substrates for any known plasma enzyme would be transferred *in vivo* by a plasma factor. This finding suggests that the diether analogs of PCs might be used to determine some of the metabolic routes for PCs *in vivo*. Ordinary PCs cannot be used because they generate additional lipid pools including fatty acid, cholesteryl ester, and lysoPC through the action of HL, LPL, and LCAT. A similar strategy might also be used to study the intracellular movement of PCs although it is not known at the present time whether lysosomal or microsomal enzymes might degrade PC ethers. Finally, if PC ethers are good physiological, nonhydrolyzable analogs of PC, they could be used to determine the absorption (if any) of PCs by the gastrointesintal tract.

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