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Effects of Human Plasma Lipid Transfer Protein on the Distribution of Lipids Between Lipoprotein Fractions of Rat Plasma*

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인체의 혈장에서 분리한 지질전이단백질이 흰쥐의 혈장 Lipoprotein의 지질분포에 미치는 영향

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흰쥐의 lipoprotein을 제거한 혈장내의 지질전이 활성도와 지진전이를 저해하는 활성도를 측정하였다. 지질전이 활성도의 측정은 방사성 동위원소가 함유된 cholesteryl ester (CE) 와 triglyceride (TG) 로 표지된 인체의 혈장 low density lipoprotein으로부터 high density lipoprotein (HDL) 으로 전이되는 방사성 동위원소의 양을 측정하여 계산하였다. 지질전이 저해 활성도는 정제한 인체 혈장 지질전이 단백질에 의한 지질전이를 저해하는 정도로서 측정하였다. 흰쥐의 혈장에는 지질전이 활성도가 거의 없으나, 지질전이를 저해하는 활성도는 존재하였다. 지질전이 저해 정도는 측정용액내의 lipoprotein 양이 증가할수록 감소하였다. 일반적으로 흰쥐의 HDL은 인체의 HDL에 비하여 CE의 함량은 높으며 TG의 함량은 낮다. 흰쥐의 혈장에 인체의 혈장으로부터 정제한 지질전이 단백질을 가하여 37℃에 24시간 두었을 때, HDL로부터 very low density lipoprotein (VLDL) 으로 CE가 이동하여 VLDL의 CE 함량이 4배나 증가하였다. 반면에 VLDL로부터 HDL_2로 TG가 이동하여 HDL_2의 TG함량은 9배나 증가하였다. 이와 같은 현상은 흰쥐의 혈장 lipoprotein이 지질전이의 기질로서의 결함은 없음을 보여준다. 따라서 지질대사에 관한 실험에서의 흰쥐에 의한 실험결과의 해석에는, 인체 혈장에 비하여 혈장에는 lipoprotein사이의 중성지질의 전이나 교환이 거의 없다는 특성이 고려되어야 한다.

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INTRODUCTION

Lipid transfer protein has been purified and characterized from human[1)3)] and rabbit[4)5)] plasma. It mediates not only exchange but net mass transfer of cholesteryl esters and triglycerides between lipoprotein fractions[6)7)]. The transfer protein promotes distribution of cholesteryl esters, which are newly formed within plasma high density lipoprotein(HDL) by the lecithin : cholesterol acyltransferase(LCAT) reaction, to less dense lipoprotein fractions[8)]. At the same time triglycerides mainly present in chylomicrons and very low density lipoproteins(VLDL), are transferred to high density lipoproteins.

It has been reported that rat plasma does not have substantial lipid transfer activity[9)10)] and addition of lipoprotein-free plasma from rats to human lipid transfer protein caused inhibition of both triglyceride and cholesteryl ester transfer[11)]. This is consistent with the fact that rat plasma has the relatively low content of cholesteryl esters in VLDL and of triglycerides in HDL[10)12)]. The lack of lipid transfer activity in rat plasma can be resulted from either the lack of the transfer protein or the inability of rat lipoproteins to serve as substrates.

In this study, rat plasma was incubated with human lipid transfer protein and effects of its incubation on the lipid composition of lipoprotein fractions were investigated.

MATERIALS AND METHODS

Preparation of rat plasma and lipoprotein-deficient plasma

Mature male Sprague-Dawley rats were fed a commercial pellet laboratory diet(Agway, Syracuse, NY). Rats were bled, after overnight fasting, by heart puncture under ether anesthesia. Blood was collected into syringes containing 0.01ml of 0.4 M EDTA, 4 %NaN_3/ml blood and kept at 4℃. Lipoprotein-deficient plasma was prepared as follows : One ml of rat plasma was adjusted to density 1.21 by adding NaBr solution(d=1.478) and centrifuged in a Beckman 50.3 rotor at 4℃ for 3×10^8 g×min The infranatant, lipoprotein-deficient plasma, was recovered from the bottom two thirds of the tube by tube slicing and the sample was dialyzed against 0.15M NaCl/0.01 % EDTA/0.02 % NaN_3 at 4 ℃ overnight.

Purification of human lipid transfer protein

Lipid trnafer protein was partially purified as described by Morton and Zilversmit[11)]. Briefly, lipoprotein-deficient plasma was prepared from citrated human plasma(Red Cross Blood Bank) by the dextran sulfate-Mn^{2+} precipitation procedure. One liter of lipoprotein-deficient plasma, adjusted to 4M NaCl with solid NaCl, was applied to a 200ml bed-volume phenyl-Sepharose CL-4B(Pharmacia Fine Chemicals) column equilibrated in 4M NaCl and washed with 900ml of 50mM Tris-HCl/150mM NaCl, pH7.4(Tris/NaCl buffer). Lipid transfer activity was eluted with H_2O containing 0.02 %NaN_3. Active fractions by lipid transfer assay were pooled, adjusted to 10mM acetate, 50 mM NaCl, pH4.5, applied to a 40ml bed-volume CM-cellulose(CM-52, Whatman) column and washed with 60ml of 10ml acetate/50mM NaCl, pH4.5. Lipid transfer activity was eluted with 10mM acetate/150mM NaCl, pH4.5. Active fractions were pooled and dialyzed against Tris/NaCl buffer(hereafter called CM-pool). All procedures were carried out at 4℃.

Assay of lipid transfer activity

Lipid transfer assays were carried out[11)] by measuring the transfer of radioactive triglyceride and cholesteryl ester from human low density lipoproteins(LDL) to human HDL. Amounts of LDL and HDL in an assay tube were equivalent to 10 μg total cholesterol in each lipoprotein fraction. Human LDL was labeled in vitro by incubating plasma with [^{3}H] triglyceride and [^{14}C] cholesteryl ester and than isolated by ultracentrifugation at

density $1.019 < d < 1.063$[10]. Unlabeled HDL was also prepared from cold plasma by ultracentrifugation at $1.063 < d < 1.21$.

Lipid transfer activity was calculated as described by Pattnaik et al[13]. Transfer activity is expressed as % kt(=100 kt) where k is the fraction of lipid transferred per unit time and t is the assay time, which unless otherwise specified, is 1.5hour. Inhibitory activity assay is expressed as percent inhibition of known transfer activity as described by Son and Zilversmit[14]. CM-pool, 12.5ul, was added in all assay tubes. The transfer activity with the addition of rat lipoprotein-deficient plasma was compared to that without lipoprotein-deficient plasma and the relative reduction of transfer activity was expressed as % inhibition.

Incubation of rat plasma with human lipid transfer protein

To investigate effects of human lipid transfer protein on the lipid composition of rat lipoproteins, the following experiment was carried out; 2.4ml of rat plasma was incubated with 2ml of CM- pool with 0.04% DTNB(5,5'-dithiobis-(2-nitrobenzoate) and 0.02% NaN_3 at pH7.4 and 37℃ for 0, 4 and 24hours. Each incubation was carried out in duplicate. To exclude the possibility of alteration in lipoproteins due to long-time incubation, rat plasma was incubated with 2ml of Tris/NaCl buffer instead of CM-poll for 24hours. After incubation for the designated time the tubes were placed in ice water and the samples were subjected to ultracentrifugation to separate individual lipoproteins.

Effects of DTNB(5,5'-dithiobis-(2-nitrobenzoate)) and PCMPS(p-chloromer-curiphenyl sulfonate) on the lipid transfer between the lipoprotein fractions with density greater and less than 1.050. Rat plasma, 1.2ml, was incubated with 0.5ml of CM-poll in the presence of either 1ml DTNB or 2 mM PCMPS at 37℃. Aliquots were taken at time, 0, 4, 8, 16 and 24hour and subjected to ultracentrifugation.

Separation and analysis of rat plasma lipoproteins

Lipoproteins of rat plasma were separated by the sequential ultracentrifugation at 4℃. VLDL ($d < 1.006$) and fraction with density between 1.006 and 1.050, in a Beckman 50.3 rotor, were obtained by centrifugation for $2.1 \times 10^8 g \times min$, and HDL_2 ($1.050 < d < 1.125$) and HLD_3 ($1.125 < d < 1.21$) for $3 \times 10^8 g \times min$. Lipids from lipoprotein samples were extracted by partitioning into hexane from 42% ethanol in water according to the method of Thompson et al[15]. Free cholesterol and cholesteryl ester were separated on precoated TLC plates with hexane/diethyl ether/acetic acid. Lipids were extracted from the silica gel with chloroform/methanol(9:1, v/v). Cholesterol was determined by the ferric chloride procedure after saponification according to Abell[17]. Triglyceride was measured by the method of Sardesai and Manning[18].

RESULTS

Although lipid transfer proteins are associated predominantly with the HDL in fasting plasma, they dissociate into the lipoprotein-deficient plasma with extensive ultracentrifugation[19]. Ultracentrifugation of rabbit plasma at density 1.21 in a Beckman 50.3 rotor at 4℃ for $3 \times 10^8 g \times min$ gave the recovery of more than 95% of lipid transfer activity in the lipoprotein-deficient plasma. By the use of the lipoprotein-deficient plasma the activities of the lipid transfer and inhibition are measured independent of lipoprotein levels in plasmas.

Lipid transfer activity in lipoprotein-deficient plasma from rats was negligible; 4.6 ± 4.0 %kt/ml from normolipidemic rats and 5.9 ± 4.6 %kt/ml from hyperlipidemic rats, as compared to approximately 300 %kt/ml and 600-1400 %kt/ml of human and rabbit lipoprotein-deficient plasmas, respectively. The presence of lipid transfer inhibitory activity in rat lipoprotein-defient plasma and an effect of substrate concentration on the relative inhibition

Table 1. Effect of substrate concentration on the relative inhibition of lipid transfer activity by lipoprotein - deficient plasma from rat

Rat lipoprotein - deficient plasma, μl	percent inhibition of transfer activity *					
	20 μg[a]		30 μg[b]		40 μg[c]	
	TG	CE	TG	CE	TG	CE
0	0 (23.4) **	0 (28.6)	0 (17.3)	0 (19.7)	0 (12.6)	0 (16.4)
26	37.5	27.7	28.3	17.2	10.2	3.8
52	45.5	34.4	33.1	23.6	19.2	15.1

* 12.5 μl of CM-pool was added in all assay tubes.

** Lipid transfer activities from incubation without rat lipoprotein-deficient plasma are shown in parentheses. (unit, % kt)

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Substrate concentrations equivalent to 20, 30 and 40 μg cholesterol in LDL plus HDL with equal amounts.

Table 2. Lipid composition of rat plasma lipoproteins after incubation with human plasma lipid transfer protein

Lipoprotein fraction	Transfer protein a)	Incubation time, hrs.	μg lipid/ml rat plasma		
			TG	CE	FC
VLDL (d < 1.006)	+	0	491	32	22
	+	4	364	81	24
	+	24	261	134	32
	-	24	484	29	33
IDL+LDL 1.006 < d < 1.150	+	0	126	77	34
	+	4	131	96	35
	+	24	147	123	37
	-	24	124	80	33
HDL_2 1.050 < d < 1.125	+	0	26	266	52
	+	4	138	237	51
	+	24	242	179	48
	-	24	28	288	49
HDL_3 1.125 < d < 1.21	+	0	22	77	11
	+	4	37	41	7
	+	24	25	14	3.5

a) As the source of transfer protein, 2 ml of CM-pool (344 μg protein corresponding to 532 % kt) was added to the incubation solution containing 2.4 ml of whole rat plasma.

of lipid transfer activity were tested and the results are shown in Table 1. Addition of 26 and 52 μl of rat lipoprotein-deficient plasma to CM-pool caused 38% and 28% reduction of triglyceride and cholesteryl ester transfer activities, respectively. The percentage reduction of lipid transfer between lipoproteins by the rat lipoprotein-deficient plasma decreased as the lipoprotein concentration increased.

Effects of human lipid transfer protein on the lipid composition of rat lipoproteins were investigated to see whether rat lipoproteins can be modified by the lipid transfer protein. The experiment was carried out as stated in methods and results are shown in Table 2. By adding DTNB into the incubation mixtures cholesterol esterification in whole plasma during incubation was blocked so that modification of the lipid composition in each lipoprotein could be ascribed only to the role of the lipid transfer protein.

As shown in Table 2, lipid distribution of rat plasma incubated without CM-pool for 24 hours was the same as that of unincubated plasma. It indicates that incubation itself did not cause any change in the lipid composition of rat plasma lipoproteins. Addition of human lipid transfer protein caused the net transfer of lipids between lipoproteins and resulted in significant changes of lipid composition: In VLDL there was a 4-fold increase in cholesteryl ester and 47% reduction in triglyceride after 24 hour incubation. In fractions with density between 1.006 and 1.050 there was 60% and 17% increases in cholesteryl ester and triglyceride, respectively. HDL_2 had a 9-fold increase in triglyceride and 33% reduction in cholesteryl ester and HDL_3 had 82% reduction in cholesteryl ester. Contents of unesterified cholesterol in all the fractions were unaffected by the incuba-

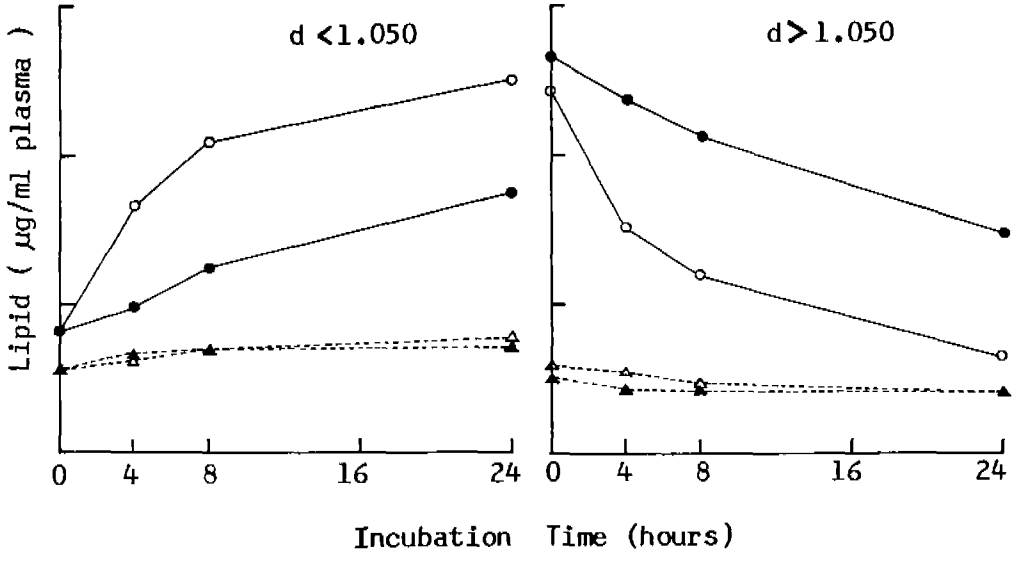

Fig. 1. Net transfer of cholesteryl ester and free cholesterol between the lipoprotein fractions with d<1.050 and d>1.050 after incubation with human lipid transfer protein in the presence of LCAT inhibitors.
As the source of human lipid transfer protein 0.5 ml CM-pool (168 μg protein, 294 %kt) was added to 1.2 ml of whole rat plasma. DTNB and PCMPS were added to the concentrations, 1 mM and 2 mM, respectively. o—o; CE, + DTNB, ●—●; CE, + PCMPS, △—△; FC, + DTNB, ▲—▲; FC, + PCMPS.

tion with the lipid transfer protein.

The differential effect of mercurial compounds such as PCMPS on triglyceride and cholesteryl ester transfer activities have been cited as evidence supporting separate triglyceride and cholesteryl ester transfer proteins[26]. PCMPS markedly inhibited the triglyceride transfer while having virtually no effect on the cholesteryl ester transfer[27,28]. On the other hand DTNB, another LCAT inhibitor, was ineffective in suppressing triglyceride transfer as well as cholesteryl ester transfer[1].

In Fig. 1 are compared effects of DTNB and PCMPS on the net transfer of cholesteryl ester between $d > 1.050$ and $d < 1.050$ fractions in rat plasma. Rat plasma was incubated with human lipid transfer protein in the presence of either 1 mM DTNB or 2 mM PCMPS at 37℃ for various time. There was not a substantial change in the contents of unesterified cholesterol in both fractions, indicating that LCAT was almost completely inhibited by both inhibitors. The net mass transfer of cholesteryl ester in the presence of PCMPS was just about an half of that with DTNB. PCMPS suppressed net transfer of cholesteryl ester as well as triglyceride between rat lipoproteins. This result indicates that two activities of cholesteryl ester and triglyceride transfers are mediated by the same protein.

DISCUSSION

Since the presence of lipid tranfer protein in plasma was recognized there have been numerous studies on the roles of the transfer protein in lipoprotein metabolism[20-23]. Nonetheless its physiological roles are still unclear. In vitro, in both human and rabbit plasmas, lipid transfer protein mediated the net mass transfer of cholesteryl ester from HDL to VLDL and of triglycerides from VLDL to HDL. This phenomenon has been reported to be linked with cholesterol efflux. Together with LCAT activity, the efflux process is associated with cholesterol esterification and cholesteryl ester transfer to acceptor lipoproteins[24]; the transfer process relieves the end-product inhibition of LCAT and consequently enhance cholesterol esterification and results in the net egress of cellular cholesterol[25]. However, rat plasma which does not have cholesteryl ester transfer activity has the rate of LCAT reaction comparable to that in human plasma[25]. Thus an interesting hypothesis that the net transfer of cholesteryl ester increases cholesterol esterification has to be tested more.

The absence of transfer activity can be ascribed to the inability of rat plasma lipoproteins to serve as substrates. When rat lipoproteins were served as substrates for the human lipid transfer protein in this study, however, they were better substrates for the net transfer of cholesteryl ester and triglyceride. This was probably due to the fact thatthe lipid distribution between lipoproteins of rat plasma was far from equilibrium state in vivo.

Rat lipoprotein-deficient plasma exerted the lipid transfer inhibitory activity and the increase of substrate concentration in the assay mixture relieved the inhibition of lipid transfer activity by rat lipoprotein-deficient plasma. It has been speculated that the lipoprotein-deficient plasma from several species, including those with and withuot significant lipid transfer activity, may contain inhibitors of lipid transfer protein. An inhibitor of cholesteryl ester and triglyceride transfer with an apparent M_r of 32000 has been purified from human plasma[4]. Apolipoproteins may also inhibit lipid transfer protein[14]. These proteins probably compete for or modify binding sites in the lipoprotein surface[29]. The result in this study supports that the mode of inhibition similar to that of human lipid thansfer inhibitor, that the inhibitor decreases lipoprotein binding of lipid transfer protein. However, it has to be tested whether inhibitors in rat lipoprotein-deficient plasma are specific for lipid transfer activity or not.

ABSTRACT

Rat lipoprotein-deficient plasma possessed a lip-

id transfer inhibitory activity when it was added to purified human plasma lipid transfer protein, while it lacked a lipid transfer activity. Incubation of whole rat plasma with partially purified human lipid transfer protein resulted in big changes in lipid distribution of rat plasma lipoproteins. There was a 4-fold increase in cholesteryl ester(CE) and 47% reduction in triglyceride(TG) in very low density lipoproteins after 24hour incubation. In high density lipoprotein 2(HDL_2) there was a 9-fold increase in TG and 33% reduction in CE. HDL_3 had 82% reduction in CE. The result indicates that the absence of the lipid transfer activity in rat plasma can be ascribed not to the inability of rat lipoproteins to serve as substrates but to the lack of the lipid transfer protein in rat plasma. Therefore, species differences in lipid transfer between lipoproteins should be taken into consideration to interpret results of studies on lipoprotein metabolism using rats.

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