Enhancement of preheparin serum lipoprotein lipase mass by bezafibrate administration

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Abstract

To clarify the clinical implication of preheparin serum lipoprotein lipase mass (preheparin LpL mass), we studied the relationships between preheparin LpL mass and serum lipids, including midband lipoproteins, which migrate between very low density lipoproteins and low density lipoproteins on polyacrylamide gel disc electrophoresis, in hyperlipidemias. And we also studied the changes of preheparin LpL mass in hypertriglyceridemic patients during bezafibrate administration, which is known to enhance LpL activity in postheparin plasma. Preheparin LpL mass correlated positively with high-density lipoprotein-cholesterol (HDL-C) \( r = 0.418, P < 0.01 \) and negatively with triglyceride (TG) \( r = -0.256, P < 0.01 \), but did not correlate with total cholesterol (TC) in 64 hyperlipidemic (type IIa, IIb and IV) patients. The midband lipoproteins were observed in 80% of hypertriglyceridemic patients (32:40). Preheparin LpL mass in midband lipoprotein-positive subjects was lower significantly than that in midband-negative subjects. When bezafibrate (400 mg/day) was administrated to 40 hypertriglyceridemic patients for 4 months, TG level significantly decreased \( (-49 \pm 7\%), P < 0.01 \), TC levels decreased \( (-11 \pm 4\%, \text{not significant}) \), and HDL-C levels increased \( (+27 \pm 4\%, P < 0.01) \). The midband lipoproteins disappeared in 95% of patients. Preheparin LpL mass significantly increased \( (+25 \pm 6\%, P < 0.0005) \). In nine patients who stopped bezafibrate, TG levels significantly increased \( (+49 \pm 7\%, P < 0.01) \) and HDL-C levels decreased \( (-27 \pm 4\%, P < 0.01) \). Preheparin LPL mass significantly decreased \( (-25 \pm 6\%, P < 0.0005) \). These results suggested that bezafibrate administration enhanced preheparin LpL mass. And it might be implicated that enhanced LpL production by bezafibrate could reflect an increase of preheparin LpL mass. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Preheparin lipoprotein lipase mass; Bezafibrate; Hypertriglyceridemia; Midband lipoproteins

1. Introduction

Lipoprotein lipase (LpL) hydrolyzes triglycerides in circulating chylomicrons and very low density lipoproteins (VLDL) on the surface of endothelial cells [1]. LpL is thought to be synthesized mainly in adipose tissues and muscles, and is transported to the luminal surface of endothelial cells [2]. The enzyme binds to the cell surface with proteoglycans via electrostatic interactions [3]. Heparin injection induces LpL activity in plasma by releasing LpL from the endothelial surface [4]. Generally, LpL activity scarcely observed in preheparin serum. Therefore, postheparin plasma has been used to analyze LpL in various lipoprotein disorders [4–6].

However, LpL mass can be detected in preheparin serum by a sensitive enzyme-linked immunoassay using a specific monoclonal antibody [7–9]. Vilella et al. [7] report that the LpL mass in preheparin plasma might be a degraded form released from the endothelial surface to the blood stream in order to be trapped by the liver. Tornvall [8] report that there is a strong positive correlation between LpL mass in preheparin plasma...
and the HDL cholesterol level as well as weak negative relations to VLDL triglyceride and cholesterol concentration in the patient suffered myocardial infarction. We [9] reported that preheparin LpL mass levels were not affected by aging and gender, but were lower in the conditions in which triglyceride catabolism was disturbed. However, the role and the clinical implications of this preheparin LpL mass remain unclear.

Bezafibrate is a hypolipidemic drug with a marked lowering effect on plasma triglycerides and a lesser extent on plasma cholesterol [10,11]. This effect is believed to be partly due to an enhancement of LpL production. And the enhancement of LpL production is clinically observed in an increase of LpL activity and mass in postheparin plasma [12].

The atherogenesis of hypertriglyceridemia has been mentioned [13,14], and one of the causes is the presence of remnant lipoproteins, which can be observed as midband lipoproteins on polyacrylamide gel disc electrophoresis [15–17]. The relationship between LpL and remnants was not fully understood.

In this paper, we studied the relationships between preheparin LpL mass and serum lipid levels in hyperlipidemias, and the relationship between midband lipoproteins and preheparin LpL mass. Then, to clarify whether the enhancement of LpL production causes an increase in preheparin LpL mass, the effect of bezafibrate administration on LpL mass in serum was studied, as well as the effects on lipid levels and the midband lipoproteins.

2. Materials and methods

2.1. Subjects

The relationship between preheparin LpL mass and serum lipid levels was studied in 64 hyperlipidemic patients (IIa: TG < 150 mg/dl and TC > 220 mg/dl; n = 18, IIb: TG > 150 mg/dl and TC > 220 mg/dl; n = 22, IV: TG > 150 mg/dl and TC < 220 mg/dl; n = 24). Forty hyperlipidemic patients (IIb; n = 18, IV; n = 22) were treated with bezafibrate (400 mg/day) for 4 months. In nine of those subjects, whose lipid levels were improved, bezafibrate administration was discontinued and the changes of lipid levels and preheparin LpL mass levels were also studied.

2.2. Blood sampling

Serum was obtained after overnight fasting. Blood was drawn from an antecubital vein into tubes. Serum was immediately separated by low-speed centrifugation at 3000 rpm for 20 min. The sample was frozen at −30°C until assay.

2.3. Enzyme-linked immunoassay of LpL mass

LpL mass was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibody against bovine milk LpL, as described by Kobayashi et al. [18]. For the assay, a kit from Daiichi Pure Chemicals (Tokyo, Japan) was used.

2.4. Plasma lipid concentrations

Total cholesterol (TC) and triglyceride concentrations were measured enzymatically, using kits from Nippon Shoji Kaisha (Osaka, Japan) in a Hitachi 7150 analyzer. HDL-cholesterol was measured by the selective inhibition method (Daiichi Pure Chemicals, Tokyo, Japan).

2.5. Polyacrylamide disc electrophoresis of serum lipoprotein

Midband lipoprotein was detected on the densitometric pattern of polyacrylamide gel disc electrophoresis [15] using Lipophor system (Jyoko, Tokyo, Japan).

2.6. Statistical analysis

Data were entered into a database designed for this study on Macintosh computer. Statistical analysis was performed using Stat View j 45. Regression analysis was performed with simple regression methods. Paired t-test was done according to non-parametric Wilcoxon signed-rank. We considered statistical significance to be a level of < 0.05.

3. Results

3.1. Correlation between preheparin LpL mass and plasma lipid levels in hyperlipidemic patients

Correlation between preheparin LpL mass and plasma lipid levels was studied in 64 hyperlipidemic patients (IIa: n = 18, IIb: n = 22, IV: n = 24). Preheparin LpL mass correlated negatively with TG levels (r = −0.256, P < 0.01) (Fig. 1A) and positively with HDL-C levels (r = 0.418, P < 0.01) (Fig. 1C). In contrast, preheparin LpL mass did not correlate with TC levels (Fig. 1B).

3.2. The incidence of midband lipoproteins in 40 hyperlipidemic patients (IIb and IV) and preheparin LpL mass in subjects with and without midband lipoproteins

The incidence of midband lipoproteins in 40 hyperlipidemic patients (IIb and IV) was 80% (32/40). Pre-
heparin LpL mass in the subjects with midband lipoproteins was significantly lower than in those without them (23.3 ± 3.4 vs 46.8 ± 9.5, P < 0.005) (Fig. 2).

3.3. Changes of serum lipid levels and preheparin LpL mass levels after administration of bezafibrate in 40 hyperlipidemic patients (IIb and IV)

TG level significantly decreased (−49 ± 7%, P < 0.01) (Fig. 3A) and HDL-C levels increased (+27 ± 4%, P < 0.01) (Fig. 3C) during bezafibrate administration. TC levels decreased (−11 ± 4%, not significant) (Fig. 3B). Preheparin LpL mass levels significantly increased (+25 ± 6%, P < 0.0005) (Fig. 3D).

3.4. Changes of midband after bezafibrate administration in 32 midband-positive patients

The midband disappeared in 95% of 32 midband-positive patients during bezafibrate administration for 3 months. A typical case is shown in Fig. 4.

3.5. Changes in serum lipid levels and preheparin LpL mass levels in the subjects who discontinued bezafibrate treatment

After cessation of bezafibrate because TG levels and TC levels lowered below normal level (TG < 150 mg/dl, TC < 220 mg/dl), TG levels significantly increased (−49 ± 7%, P < 0.01) (Fig. 5A) and HDL-C levels decreased (−49 ± 7%, P < 0.01) (Fig. 5C). TC levels did not significantly change (Fig. 5B). Preheparin LpL mass levels significantly decreased after cessation of bezafibrate (−49 ± 7%, P < 0.01) (Fig. 5D).

3.6. Correlation between the changes in preheparin LpL mass and the changes in plasma lipid levels in 40 hyperlipidemic patients after 3 months of administrations (Figures were not shown)

There was slightly negative correlation between the changes in preheparin LpL mass and changes in TG concentrations, but it was not significant (r = −0.004, P = 0.98). However, there was significant positive correlation between the changes in preheparin LpL mass and changes in HDL-C concentrations (r = 0.28, P < 0.05).

4. Discussion

The studies on the function of LpL have utilized plasma after heparin injection, because there is little LpL activity in preheparin plasma and LpL activity appears after heparin injection [4–6]. However, recently it is reported that LpL mass exists in plasma before heparin injection [7–9]. Zambon et al. report [19] that the preheparin LpL is associated with cholesterol-rich lipoproteins such as LDL and HDL. But the clinical implication of preheparin LpL mass remains unclear.

In our study, preheparin LpL mass positively correlated with HDL-C levels and negatively with TG levels (Fig. 1A,C). These results are consistent with the idea that preheparin LpL mass could reflect some amount of
working LpL in the whole body, because LpL itself hydrolyzes triglycerides, resulting in a decrease of serum triglyceride level and an increase of HDL-C.

Preheparin LpL mass level in the subjects with midband lipoproteins were lower than in the subjects without them. The mechanism by which midband lipoproteins appear, is supposed to be due to impaired triglyceride-rich lipoprotein catabolism by LpL insufficiency and/or impaired clearance by remnant receptor. Then, low LpL mass in midband-positive patients might be compatible with the view that preheparin LpL mass reflects some amount of working LpL in a whole body.

Bezafibrate reduced TG levels and rose HDL-C levels (Fig. 3). Those effects could be explained by enhancing LpL production with bezafibrate administration. This is already reported by several investigators [12,20]. Interestingly, preheparin LpL mass was significantly elevated by bezafibrate administration (Fig. 3D). And after its cessation, preheparin LpL mass decreased (Fig. 5D). These results indicated that bezafibrate increased preheparin LpL mass.

As for the mechanism by which bezafibrate enhanced preheparin LpL mass, the precise is unclear, but the following could be supposed; bezafibrate activates the peroxisome proliferator-activated receptor (PPAR)\(\alpha\) [21], which increases expression of LpL. The enhanced LpL expression would cause an increase in the amount of LpL bound to the surface of endothelial cells. After working, the LpL would undergo denaturation, and would be detached to the blood stream. Resultantly, preheparin LpL mass would increase.

As for the postheparin LpL mass, it is unclear what percentages of LpL separate from whole surface of vascular endothelial cells by heparin injection. Considering that postheparin LpL mass is affected by several
factors such as a dose of heparin, a time after injection and the circulation, it cannot be denied that postheparin LpL mass contains artificial factor.

The midband lipoproteins were disappeared by bezafibrate (Fig. 4). Simultaneous observations of an increase in preheparin LpL mass and disappearance of midband might suggest that preheparin LpL mass is involved in the clearance of remnant lipoproteins. Biesiegel et al. [22] report that LpL itself could mediate remnant uptake and LpL can act as a ligand for the LDL-receptor related protein. On the other side, Huff et al. [23] report that inactive LpL does not enhance remnant uptake into Hep G2 cells using denatured bovine milk LpL. But, the studies using actual human preheparin LpL mass will be required to identify the function of the preheparin LPL mass as a ligand for remnant receptor.

In summary, bezafibrate administration enhanced preheparin LpL mass. The result might implicate that enhanced LpL production in a whole body reflects an increase in preheparin LpL mass.

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