Increased Serum Triglyceride Clearance and Elevated High-Density Lipoprotein 2 and 3 Cholesterol During Treatment of Primary Hypertriglyceridemia with Bezafibrate

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ABSTRACT

Background: Hypertriglyceridemia accompanied by low levels of high-density lipoprotein cholesterol (HDL-C) is a risk factor for coronary artery disease. High-density lipoprotein 2 (HDL₂) and 3 (HDL₃) are believed to suppress the progress of atherosclerosis through reverse cholesterol transport. As a result, peripheral tissues can be protected against excessive accumulation of cholesterol. Although bezafibrate is known to accelerate the increase of HDL-C, results are not standardized regarding increases of HDL₃ and HDL₂ subfractions.

Objective: This study assessed the effects of bezafibrate on serum triglyceride (TG) fractional clearance rate (K_2) and HDL₂ and HDL₃ cholesterol (HDL₂-C and HDL₃-C, respectively) levels in patients with primary hypertriglyceridemia (serum TG ≥ 150 mg/dL).

Methods: Outpatients with primary hypertriglyceridemia were enrolled in this 8-week study conducted at the Third Department of Internal Medicine, Nagoya City University Hospital (Nagoya, Japan). Oral bezafibrate was administered at a dose of 400 mg/d (200-mg tablet BID, morning and evening) for 8 weeks. After 8 weeks, serum levels of total cholesterol (TC), TG, HDL-C, HDL₂-C, and HDL₃-C were measured. A fat emulsion tolerance test to assess K_2 and measurements of plasma lipoprotein lipase (LPL) mass, LPL activity, and hepatic triglyceride lipase (HTGL) activity in postheparin plasma were performed before bezafibrate administration and after the course of treatment.

Results: Sixteen patients (10 men, 6 women; mean [SD] age, 54 [12] years [range, 30–69 years]; mean [SD] body mass index, 23 [2] kg/m²) entered the

study. The following findings were observed in male and female patients after 8 weeks of treatment. A statistically significant reduction was observed in mean serum TG level ($P < 0.01$). Significant increases were seen in HDL-C, HDL₂-C, and HDL₃-C (all $P < 0.01$), K₂ ($P < 0.01$), and in plasma LPL mass ($P < 0.01$) and LPL activity ($P < 0.05$). TC level and HTGL activity did not change significantly. No adverse effects related to the use of bezafibrate were documented.

Conclusions: In this study, bezafibrate treatment resulted in significant decreases in serum TG level and significant increases in HDL₂-C and HDL₃-C levels and plasma LPL mass and activity. We hypothesize that bezafibrate may increase HDL₃-C by promoting TG-rich lipoprotein catabolism and may increase HDL₂-C by promoting the conversion of HDL₃ to HDL₂. (*Curr Ther Res Clin Exp.* 2003; 64:697–706) Copyright © 2003 Excerpta Medica, Inc.

Key words: bezafibrate, serum triglyceride clearance, high-density lipoprotein 2, high-density lipoprotein 3, lipoprotein lipase, hepatic triglyceride lipase.

INTRODUCTION

Hypertriglyceridemia accompanied by low levels of high-density lipoprotein cholesterol (HDL-C) is a risk factor for coronary artery disease (CAD).^{1,2} It has been suggested by some investigators that adenosine triphosphate-binding cassette transporter A1 (ABCA1) has a role in the transfer of cellular cholesterol to apolipoprotein (apo) A-I and the assembly of HDL-C.^{3–5} However, the exact role of ABCA1 in this reaction has not been fully identified. Conversely, HDL-C production in vivo is thought to occur in conjunction with the catabolism of triglyceride (TG)-rich lipoproteins (very-low-density lipoprotein [VLDL] and chylomicrons) by lipoprotein lipase (LPL).^{6,7} High-density lipoprotein (HDL) (density, 1.063–1.210 g/mL) comprises the subfractions HDL₂ (density, 1.063–1.125 g/mL) and HDL₃ (density, 1.125–1.210 g/mL). The size of nascent HDL particles present in the HDL₃ subfraction is increased by accepting free cholesterol from peripheral tissue, and the particles are then converted to HDL₂. HDL₂ functions by transferring the cholesterol received from peripheral tissues. HDL₂ contains apo E, which has high ligand activity, to the low-density lipoprotein (LDL) receptor of hepatic cells. It is thought that HDL₂ transfers extracellular cholesterol to the liver either via scavenger receptor class B1 or via the LDL receptor.⁸ HDL₃ and HDL₂ are believed to suppress the progress of atherosclerosis through reverse cholesterol transport, by which peripheral cell cholesterol returns to the liver for catabolism. As a result, peripheral tissues can be protected against excessive accumulation of cholesterol.

Hepatic triglyceride lipase (HTGL) influences HDL density by hydrolyzing the TG in HDL₂ in a reverse conversion of HDL₂ to HDL₃.⁹ Although bezafibrate is known to accelerate the increase of HDL-C, results are not standardized regarding increases of HDL₃ and HDL₂ subfractions.¹⁰ Accelerated production and a processing disorder of TG-rich lipoproteins are believed to be factors

causing hypertriglyceridemia. Fat emulsion tolerance tests (FETTs) using an artificial fat emulsion (Intralipid®, Fresenius Kabi AB, Uppsala, Sweden) were performed on patients with primary hypertriglyceridemia (serum TG ≥ 150 mg/dL) to measure the serum TG fractional clearance rate (K_2) to manage treatment.^{11,12} The K_2 value has been shown to correlate closely with LPL mass and LPL activity in postheparin plasma.¹³ We previously reported that bezafibrate {propanoic acid (2-(4-(2-((4-chlorobenzoyl)amino)ethyl)phenoxy)-2-methyl))}, a derivative of clofibrate that improves lipid metabolism, accelerates clearance of TG-rich lipoprotein, thereby lowering serum TG and elevating HDL-C.¹⁴

In the present study, the effects of bezafibrate on K_2 , HDL₃-C, and HDL₂-C were assessed in patients with primary hypertriglyceridemia.

PATIENTS AND METHODS

Patients with primary hypertriglyceridemia attending the outpatient clinic of the Third Department of Internal Medicine, Nagoya City University Hospital (Nagoya, Japan) were recruited for this study. All patients underwent a screening protocol consisting of medical history, physical examination, and laboratory tests (fasting plasma glucose, serum lipids, renal profile, and liver and thyroid function tests). Exclusion criteria were as follows: diabetes mellitus; renal, hepatic, or endocrine disease; any other cause of secondary hypertriglyceridemia; febrile illness; neoplasm; inflammatory disease; and immunosuppressive therapy (including use of steroids and vitamin supplements). All patients participated in this study after giving their written informed consent. The patients did not take a fibrate or any lipid-lowering drug or other medication before the trial.

Six months before bezafibrate administration, all patients had been placed on a restricted diet of ~30 to 35 kcal/kg of ideal body weight per day. Total calories consisted of 25% fat, 55% carbohydrates, and 20% protein. Cholesterol intake was <300 mg/d. The ratio of polyunsaturated to saturated fatty acid was 1:1. The regimen was unchanged throughout the study. Patients were instructed to maintain their level of physical activity, their smoking habits, and their typical alcohol intake.

Bezafibrate was administered orally at a dose of 400 mg/d (200-mg tablet BID, morning and evening) for 8 weeks. FETT to assess K_2 values, measurement of LPL mass and activity, and measurement of HTGL activity in postheparin plasma were performed before bezafibrate administration and after the 8-week course of treatment using blood samples obtained after overnight fasting.

Patients were seen at week 4 of treatment for clinical examination and biologic assessment. They returned their leftover pills and were then given their supply of drugs for the next 4 weeks. Compliance was assessed by means of a pill count at 4 and 8 weeks.

Serum total cholesterol (TC) and TG were determined using enzymatic methods. HDL, HDL₂, and HDL₃ were isolated from the serum by sequential flotation

ultracentrifugation according to the method of Havel et al,¹⁵ after which cholesterol levels were determined using enzymatic methods. FETT was performed after blood samples had been collected following fasting for 14 hours in the morning for measurement of the aforementioned serum lipids, HDL-C, HDL₂-C, and HDL₃-C. Two hours later, blood samples were obtained before and 10 minutes after rapid IV administration of heparin sodium (30 IU/kg body weight) for measurement of plasma LPL mass and activity and HTGL activity.

FETT was performed using an artificial 10% fat emulsion (0.25 mL/kg body weight) administered IV over 90 seconds.¹¹ The midpoint of the IV administration (45 seconds) was defined as the 0 time point, and blood samples were collected at 3, 5, 7, 9, 11, 14, 17, and 20 minutes. Thereafter, each blood sample was centrifuged at 4°C and 1800g for 30 minutes to obtain the serum. Serum samples obtained at each time point were divided in half, and each portion was diluted 100-fold with physiological saline. The light-scattering index (LSI) was determined for each time point with a nephelometer (model DN-2110, Kyoto Daiichi Kagaku, Kyoto, Japan) to obtain the mean value. The wavelength used with this nephelometer was ≥ 610 nm. The LSI is expressed in terms of the value obtained with the specially made optical glass standard with which this instrument is equipped. Using the logarithmic value of the mean LSI of samples at each time point minus the mean LSI of samples obtained before fat emulsion loading, we calculated the regression coefficient ($-b$) of a steadily declining LSI curve by the method of least squares. This value was used to indicate the K_2 of the fat emulsion. As an index of the reliability of b , the Student t value was obtained from b/S_b , where S_b represents the standard error of b . The reliability of K_2 was determined from the t value; if the t value was >5 , the confidence interval of K_2 was considered to be small. The t values of K_2 of the patients in this study were >5 .

The postheparin plasma LPL mass was measured with an LPL kit (MARKIT-F LPL, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) using a sandwich-enzyme immunoassay system according to the manufacturer's instructions.¹⁶ The postheparin plasma LPL activity and HTGL activity were measured according to the method of Nozaki et al.¹⁷

Tolerability was assessed by one of the authors (N.S.) using patient interview.

Statistical Analysis

Therapeutic efficacy was determined using the paired t test, with a value of $P < 0.05$ being statistically significant. Excel Statistical Program file (Microsoft Corporation, Redmond, Washington) was used for statistical analysis.

RESULTS

Sixteen patients (10 men; 6 women; mean [SD] age, 54 [12] years; mean [SD] body mass index, 23 [2] kg/m²) entered the study (Table I).

Table I. Baseline demographic and clinical characteristics of study patients (N = 16).

Patient No.	Age, y	BMI, kg/m ² (NV, 19.8–24.2)	TG, mg/dL (NV, 50–<150)	TC, mg/dL (NV, <200)	HDL ₂ -C, mg/dL (NV, 16–73)	HDL ₃ -C, mg/dL (NV, 13–25)	K ₂ , %/min (NV, 4–27.6)
Men							
1	61	24	153	187	21	14	7.3
2	59	25	237	208	11	13	11.2
3	48	26	294	203	26	14	5.0
4	54	23	497	238	25	17	5.6
5	30	22	250	385	20	19	6.6
6	37	26	313	250	24	18	7.6
7	33	23	774	243	21	18	2.1
8	44	21	155	257	40	31	12.3
9	50	24	1372	309	20	12	4.0
10	57	26	707	289	18	20	4.0
Mean (SD)	47 (11)	24 (2)	475 (383)	257 (58)	23 (7)	18 (5)	6.6 (2.7)
Range	30–61	21–26	153–1372	187–385	11–40	12–31	2.1–12.3
Women							
11	67	24	172	305	30	25	11.2
12	69	24	222	250	18	19	5.2
13	66	21	585	210	19	12	4.3
14	55	24	306	161	18	14	4.5
15	63	21	249	205	34	15	8.0
16	64	19	204	354	46	20	12.6
Mean (SD)	64 (5)	22 (2)	290 (152)	248 (71)	28 (11)	18 (5)	7.6 (2.8)
Range	55–69	19–24	172–585	161–354	18–46	12–25	4.3–12.6

BMI = body mass index; NV = normal value; TG = triglycerides; TC = total cholesterol; HDL₂-C = high-density lipoprotein 2 cholesterol; HDL₃-C = high-density lipoprotein 3 cholesterol; K₂ = fractional clearance rate.

The following findings were observed in both male and female patients. After 8 weeks of treatment with bezafibrate, the mean serum TG level decreased significantly ($P < 0.01$) and the plasma K_2 value increased significantly ($P < 0.01$), but the TC level did not change significantly. Serum HDL₂-C and HDL₃-C levels increased significantly (all $P < 0.01$) (Figure). Plasma LPL mass and LPL activity in the postheparin plasma were significantly elevated ($P < 0.01$ and $P < 0.05$, respectively). However, postheparin plasma HTGL activity did not change significantly (Tables II and III).

None of the patients withdrew from the study. All patients took 100% of the estimated dosage. No adverse effects related to the use of bezafibrate were documented.

DISCUSSION

Low serum HDL-C levels and high serum TG levels are common in patients with CAD.^{1,2} Our results indicate bezafibrate decreases serum TG and increases HDL-C, suggesting that bezafibrate has antiatherosclerotic activity. Nicoll et al¹⁸ reported a strong positive correlation between the VLDL apolipoprotein B fractional catabolic rate and the FETT K_2 value. Similarly, Rössner et al¹⁹ reported a positive correlation between the endogenous TG fractional removal rate and K_2 . Furthermore, TG-rich lipoprotein metabolism can be inferred if the FETT K_2 value is known.

A positive correlation exists between the VLDL-TG fractional catabolic rate and HDL-C in humans,²⁰ and HDL production in vivo is thought to occur mainly in conjunction with the catabolism of TG-rich lipoproteins.^{6,7}

Figure. Mean (SD) (A) high-density lipoprotein 2 cholesterol (HDL₂-C) and (B) high-density lipoprotein 3 cholesterol (HDL₃-C) levels before and after 8 weeks of treatment with bezafibrate. * $P < 0.01$ versus before treatment.

Table II. Mean (SD) laboratory findings in male patients (n = 10) before and after 8 weeks of treatment with bezafibrate.

Component	Normal Value	Before Treatment	After Treatment	<i>p</i> *
TG, mg/dL	50–<150	475 (383)	272 (239)	<0.01
TC, mg/dL	<200	257 (58)	249 (48)	NS
HDL-C, mg/dL	40–86	40 (12)	47 (12)	<0.01
HDL ₂ -C, mg/dL	16–73	22 (7)	25 (8)	<0.01
HDL ₃ -C, mg/dL	13–25	18 (6)	22 (5)	<0.01
K ₂ , %/min	4.0–27.6	6.2 (2.7)	9.1 (2.8)	<0.01
LPL activity, μ mol/mL·min	0.193–0.199	0.46 (0.07)	0.51 (0.05)	<0.05
LPL mass, ng/mL	164–284	239 (84)	296 (36)	<0.01
HTGL, μ mol/mL·min	5.90–11.70	0.31 (0.06)	0.33 (0.09)	NS

TG = triglycerides; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; HDL₂-C = high-density lipoprotein 2 cholesterol; HDL₃-C = high-density lipoprotein 3 cholesterol; K₂ = fractional clearance rate; LPL = postheparin plasma lipoprotein lipase; HTGL = postheparin plasma hepatic triglyceride lipase.

*Student *t* test.

In one study,²¹ HDL-C and both HDL₃-C and HDL₂-C were inversely related to the risk for myocardial infarction. HDL₃ and HDL₂ play different roles in reverse cholesterol transport and represent different stages in the overall metabolism of HDL. In this study, administration of bezafibrate increased HDL₃-C, HDL₂-C, and HDL-C. Possible mechanisms of the increase in HDL₃-C and HDL₂-C subfractions are described below. It is known that bezafibrate activates the peroxisome

Table III. Mean (SD) laboratory findings in female patients (n = 6) before and after 8 weeks of treatment with bezafibrate.

Component	Normal Value	Before Treatment	After Treatment	<i>p</i> *
TG, mg/dL	50–<150	290 (152)	167 (95)	<0.01
TC, mg/dL	<200	248 (71)	224 (26)	NS
HDL-C, mg/dL	40–86	45 (14)	59 (15)	<0.01
HDL ₂ -C, mg/dL	16–73	27 (11)	36 (12)	<0.01
HDL ₃ -C, mg/dL	13–25	18 (5)	23 (5)	<0.01
K ₂ , %/min	4.0–27.6	7.0 (2.8)	10.8 (2.7)	<0.01
LPL activity, μ mol/mL·min	0.193–0.199	0.44 (0.06)	0.49 (0.04)	<0.05
LPL mass, ng/mL	164–284	188 (65)	263 (32)	<0.01
HTGL, μ mol/mL·min	5.90–11.70	0.34 (0.09)	0.29 (0.08)	NS

TG = triglycerides; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; HDL₂-C = high-density lipoprotein 2 cholesterol; HDL₃-C = high-density lipoprotein 3 cholesterol; K₂ = fractional clearance rate; LPL = postheparin plasma lipoprotein lipase; HTGL = postheparin plasma hepatic triglyceride lipase.

*Student *t* test.

proliferator-activated receptor, which increases expression of the LPL enzyme protein and LPL activity.²² Previously, we reported a significant correlation between K_2 and postheparin plasma LPL mass and activity.¹³ In the current study, the K_2 value and LPL mass and activity in postheparin plasma increased. From these results, it is supposed that TG-rich lipoprotein catabolism is accelerated, resulting in increased production of HDL₃ by bezafibrate administration.

A conversion factor in plasma acts on HDL₃ to promote conversion of HDL₃ to HDL₂.²³ HTGL hydrolyzes the TG in HDL₂, converting HDL₂ to HDL₃ and reducing HDL₂ density. However, our results did not show a significant change in HTGL activity, which suggests that the increase in HDL₂-C is a consequence of bezafibrate increasing the activity of the factor that promotes conversion of HDL₃ to HDL₂, thereby accelerating the conversion. Conversely, HDL₃ removes cholesterol from peripheral tissues. HDL₃ converts to HDL₂ after receiving cholesterol from peripheral tissues. After this phenomenon, HDL₂ directly transports the cholesterol of peripheral tissues to the liver. Because HDL₂-C increases with bezafibrate administration, it is supposed that bezafibrate accelerates this process. Release of cellular cholesterol involves at least 2 distinct mechanisms: (1) the physicochemical diffusion of cholesterol from the cell surface and (2) the apolipoprotein-mediated pathway that releases cholesterol and phospholipid to generate new HDL particles.⁵ However, the effect of bezafibrate on the release of cellular cholesterol by these 2 distinct mechanisms is not known and is a subject of further research in our laboratory. Bezafibrate is known to reduce the progression of CAD.²⁴ An association exists between increased plasma fibrinogen levels and the onset and progression of CAD.^{25,26}

Bezafibrate has been reported to decrease plasma fibrinogen level by ~20%.²⁷ It is believed that systemic inflammation may contribute to the increased cardiovascular risk in patients who have hypertriglyceridemia. Bezafibrate therapy in hypertriglyceridemia has been shown to significantly reduce the production capacity of tumor necrosis factor- α and interleukin 6, as well as levels of C-reactive protein and fibrinogen.²⁸ Meanwhile, it is reported that HDL has anti-inflammatory, antiaggregatory, anticoagulant, and profibrinolytic activities.²⁹ Also, HDL₂ and HDL₃ have abilities against the formation of peroxidatively modified LDL.^{30,31} From these reports, it can be supposed that the development of atherosclerosis may be suppressed because of a reduction of systemic inflammation or through other mechanisms involving increases in both HDL₂-C and HDL₃-C by bezafibrate administration.^{24,32}

CONCLUSIONS

In this study, bezafibrate treatment resulted in significant decreases in serum TG level and significant increases in HDL₂ and HDL₃ cholesterol levels and plasma LPL mass and activity. We hypothesize that bezafibrate may increase HDL₃-C by promoting TG-rich lipoprotein catabolism and may increase HDL₂-C by promoting the conversion of HDL₃ to HDL₂.

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