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ORIGINAL ARTICLE

EFFECT OF FENOFIBRATE ON LIPID LEVELS OF TYPE 2 DIABETIC PATIENTS WITH DYSLIPIDEMIA

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Abstract The effect of fenofibrate in type 2 diabetic patients with dyslipidemia was examined. Thirteen patients with type 2 diabetes mellitus and mixed hyperlipidemia; serum total cholesterol (TC) > 220 mg/dl and/or triglyceride (TG) > 150 mg/dl, were included in the study. Fenofibrate was administered at daily dose from 200 to 300 mg, and following items were implemented just prior to, at 3 months after, and at 6 months after the administration: amount of serum lipid, electrophoresis of lipoprotein by the method of polyacrylamide gel electrophoresis (PAGE), quantification of cholesterol (RLP-C) and triglyceride (RLP-TG) in remnant-like particle. TC significantly decreased at 6 months after administration ($p<0.05$), while TG at 3 and 6 months after administration was significantly lowered ($p<0.05$). Concentration of RLP-C that was 16.3 mg/dl before administration of fenofibrate decreased with statistical significance to attain 6.2 mg/dl and 7.2 mg/dl in 3 and 6 months after administration, respectively ($p<0.01$). In the case of RLP-TG, the concentration of 92.2 mg/dl before administration was observed to be lowered with time with statistical significance to reach 28.7 mg/dl and 32.7 mg/dl in 3 and 6 months, respectively ($p<0.01$). The low-density lipoprotein (LDL) particle size significantly increased at 6 months after administration ($p<0.05$). In all cases the remnant lipoprotein was observed on PAGE before administration, the bands disappeared or decreased in 8 cases by the treatment with fenofibrate. It was suggested that fenofibrate lowered RLP-C and RLP-TG, and improved the LDL particle size in type 2 diabetics with dyslipidemia.

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Key words: small, dence LDL; remnant lipoprotein; remnant-like particle.

原 著

脂質代謝異常を呈する2型糖尿病患者に対するフェノフィブラートの効果

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抄録 高脂血症を有する2型糖尿病患者に対するフェノフィブラートの臨床効果を検討した．フェノフィブラート1日200から300 mgを投与し，投与前，3ヵ月後，6ヵ月後について，脂質検査を行った．血清総コレステロール（TC）は，6ヵ月目で，中性脂肪（TG）は3ヵ月，6ヵ月とも有意の低下を認めた．レムナント様粒子コレステロール（RLP-C）は投与前16.3 mg/dlから，3ヵ月で6.2，6ヵ月で7.2 mg/dlと低下を認めた．RLP-TGは投与前92.2 mg/dlから3ヵ月で28.7，6ヵ月で32.7 mg/dlと著明に低下した．低比重リポ蛋白（LDL）の粒子サイズは6ヵ月目で増加した．ポリアクリルアミド電気泳動（PAGE）では，8例にミッドバンドの改善がみられた．フェノフィブラートが2型糖尿病患者のRLP-C，RLP-TG，LDL粒子サイズを改善し有効であることが示された．

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キーワード: 小型高比重LDL; レムナントリポ蛋白; レムナント粒子．

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Introduction

In many epidemiological researches, it has been clarified that diabetics frequently develop diseases of atherosclerosis such as coronary heart diseases and cerebrovascular diseases[1]. Dyslipidemia in diabetes has been thought to be one of the reasons for the development of such complication[2]. Hypertriglyceridemia in type 2 diabetics is observed with high frequency, which is attributed to the increase in very low-density lipoprotein (VLDL). In addition, increase in the concentrations of remnant lipoprotein and the small, dense LDL[3], and decrease in high-density lipoprotein cholesterol (HDL-C) in the blood[4] are known to be as qualitative abnormalities. Fenofibrate has been used in our country since 1999, and is the drug of fibrate analogues. Fenofibrate possesses the activities to decrease in the amounts of total cholesterol (TC) and LDL cholesterol (LDL-C) in addition to the activities to decrease in the amounts of triglyceride (TG) and to increase in HDL-C[5] accordingly since fenofibrate exerts intensive efficacy toward improvement of serum lipids, this drug has widely been used in clinical fields. This research especially considered the influence of fenofibrate on the plasma levels of remnant fraction which closely concerned with atherosclerosis, in patients with type 2 diabetes.

Materials and Methods

Subjects

The 13 subjects enrolled in this study were outpatients in our university hospital (The Third Internal Medicine) and were categorized to the type 2 diabetics who were subjected to mixed hyperlipidemia (serum total cholesterol: 220 mg/dl or higher, triglyceride: 150 mg/dl or higher) in spite of dietary therapy and life guidance. The subjects were 8 males and 5 females at 53.4 ± 15.3 years old and whose BMI was 25.9 ± 4.0 kg/m^2. The contents of medical treatment for diabetes were as follows: only dietary therapeutics: 4 subjects, administration of oral anti hyperglycemic drugs: 4 subjects, insulin therapeutics: 5 subjects. The complications of diabetes were as follows: no diabetic retinopathy (5 subjects), simple retinopathy (3 subjects), proliferative retinopathy (5 subjects), and persistent proteinuria (8 cases). The cases that were complicated by severe liver damage or renal diseases giving serum creatinine at 1.2 mg/dl or higher, and cases with acute coronary diseases and cerebrovascular diseases were excluded from the subjects in the present study. The informed consent was obtained from the patients after the explanation of the study protocol.

Methods

All the subjects were administered with fenofibrate once a daily dose of 200 mg or 300 mg after intake of breakfast. With respect to the subjects who were already receiving anti-diabetic agents or anti-hyperlipidemic agents, the kinds of such drugs and doses were in principle not changed during the course of therapy.

Before administration, and 3 and 6 months after the administration of fenofibrate, the blood was collected under the fasting conditions. TC, TG, and HDL-C in the serum were quantificated by the enzyme assay method with an auto analyzer. Amounts of LDL-C were calculated according to an equation of Friedewald's. HbA1c was determined by HPLC method. The amounts of cholesterol and triglyceride in remnant-like particle (RLP-C and RLP-TG) were measured by affinity chromatography as follows; after 300 μl of mixed gel of monoclonal anti-apoB100 antibody and monoclonal anti-apoA-I antibody were added with 5 μl of the serum, the mixture

was gently stirred for 120 min at room temperature followed by centrifugation. Amounts of cholesterol and TG were determined in the supernatant thus obtained. Electrophoresis of lipoprotein was carried out by the method of polyacrylamide gel electrophoresis (PAGE) (Lipophor system, Joko)[6]. As index of particle size of LDL, LDL relative migration ratio (LDL migration index, hereinafter abbreviated as LDL-MI) in PAGE was determined[7]. The LDL-MI was calculated according to a following equation:

$$\text{LDL-MI} = \frac{\text{Distance from peak of VLDL to peak of LDL}}{\text{Distance from peak of VLDL to peak of HDL}}$$

Mishima et al. reported that LDL particle size determined by density gradient gel electrophoresis gave significant negative correlation with LDL-MI[7].

The measured values were expressed as mean ± standard error (S.E.), and examined by Wilcoxon signed ranks test with a significant level of 0.05 ($p<0.05$).

Results

1. Changes of serum lipids (Table 1)

The concentrations of TC lowered with time after administration of fenofibrate, and were 237 ± 12 mg/dl, 226 ± 15 mg/dl, and 204 ± 11.4 mg/dl before, and in 3 and 6 months after administration, respectively. The concentration in 6 months after administration gave significant difference ($p<0.05$) compared with that before administration. The concentrations of TG were 331 ± 68 mg/dl prior to administration, whereas the concentrations in 3 and 6 months after administration decreased to 158 ± 17 mg/dl and 116 ± 24 mg/dl, respectively. The latter two concentrations were significantly lower than the former ($p<0.05$). The concentrations of HDL-C before, and in 3 and 6 months after administration were 48.1 ± 3.0 mg/dl, 54.5 ± 2.3 mg/dl, and 51.2 ± 2.5 mg/dl, respectively; the value in 3 months was significantly higher than that before administration ($p<0.05$). The concentration of LDL-C was 133 ± 12 mg/dl before administration. When this concentration was compared with the respective concentrations of 129 ± 11 mg/dl and 127 ± 12 mg/dl in 3 and 6 months after administration, tendency of decreasing was observed although the changes were statistically insignificant.

2. Changes of RLP-C and RLP-TG (Fig. 1 [A] and [B])

Concentration of RLP-C was 16.3 ± 4.4 mg/dl before administration of fenofibrate, while both concentrations in 3 and 6 months after administration decreased with statistical significance to attain 6.2 ± 0.9 mg/dl and 7.2 ± 1.1 mg/dl ($p<0.05$), respectively. In the case of RLP-TG, the concentration of 92.2 ± 31.9 mg/dl before administration was observed to be lowered with time with statistical significance ($p<0.01$) to reach 28.7 ± 5.1 mg/dl and 32.7 ± 7.6 mg/dl in 3 and 6 months after administration, respectively.

Table 1 Changes of serum lipids before and after fenofibrate administration

		month		
		0	3	6
TC	(mg/dl)	237±12	226±15	204±11*
TG	(mg/dl)	331±68	158±17*	116±24*
HDL-C	(mg/dl)	48.1±3.0	54.5±2.3*	51.2±2.5
LDL-C	(mg/dl)	133±12	129±11	127±12

Data are mean±S.E. *$p<0.05$ v.s. 0 month. TC; total cholesterol, TG; triglyceride, HDL-C; high-density lipoprotein cholesterol.

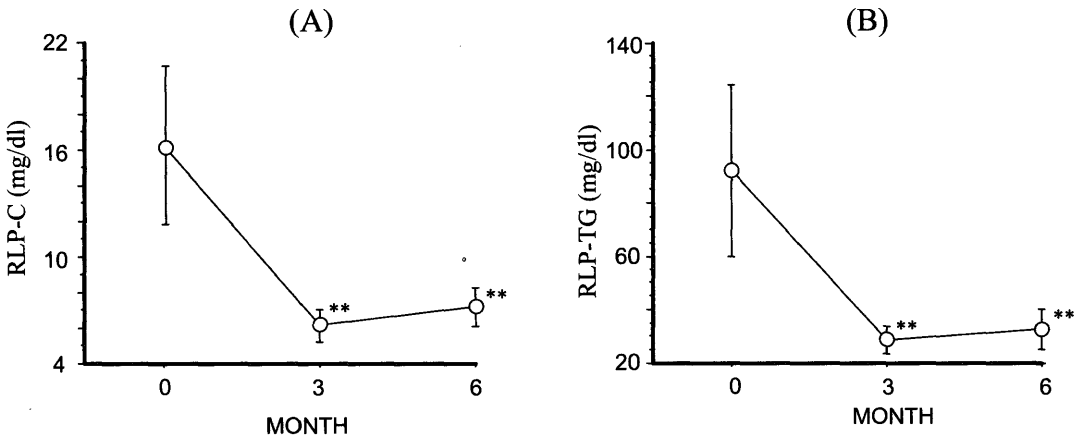

Figure 1 Changes of RLP-C and RLP-TG.
Effects of fenofibrate on serum RLP-C (A) and RLP-TG (B) levels were illustrated. Values are expressed as mean ± S.E. $**p<0.01$ compared with baseline levels.

3. Change of LDL particle size

LDL-MI before administration, and in 3 and 6 months after administration of fenofibrate were 0.37 ± 0.01, 0.35 ± 0.01, and 0.34 ± 0.01, respectively. LDL-MI significantly decreased in 6 months after administration ($p<0.05$). The results suggested that administration of fenofibrate caused the increase in LDL particle sizes. In all cases mid-bands were observed on PAGE before administration, whereas the bands disappeared or decreased in 8 cases by the treatment with fenofibrate.

4. Influence on glucose tolerance

Although HbA1c that was 7.2% ± 0.4% before administration gave tendency to increase to achieve 7.5% ± 0.4% in 3 months and 7.6% ± 0.5% in 6 months after administration of fenofibrate, the increase was statistically insignificant. No apparent side effects were observed during the course of administration of fenofibrate.

Discussion

The diabetes, especially type 2 diabetes complicate dyslipidemia such as hypertriglyceridemia, low HDL cholesterolemia at high frequency, and these are important risk factors for the diabetic macroangiopathy together with obesity, hypertension, and smoking[1-2]. Since hyperlipemia concurred with diabetes is influenced by the controlled state of blood glucose and the frequency has been generally considered to be in the range from 20% to 70%. For the purpose of improvement of these disorders of lipoprotein metabolism, many types of anti-hyperlipidemic agents have been developed and used long before. Fournier Inc. in France launched fenofibrate on market in 1975 that is one of the anti-hyperlipidemia categorized in fibrate analogues. Fenofibrate has been now used in 80 countries or more in the world, and was placed on sale as Lipantil® since 1999 in Japan. Fenofibrate is recognized to be an useful therapeutic agent for Type IIb and Type IV hyperlipemia (WHO classification); because it possesses strong activity to decrease the blood concentration of VLDL and to increase of HDL-C[8-10]. Various actions have been reported for fenofibrate, and as the action mechanism of this drug it is clarified that fenofibrate exerts its activity through peroxisome proliferator-activated receptor α (PPAR α). Fenofibrate activates

PPAR α by acting as its ligand [11] to lead to the increase in the activity of lipoprotein lipase (LPL)[12], the suppression of expression of apo C-III that inhibits LPL activity[13], and the acceleration of β-oxidation of fatty acids in the mitochondria[14]. In addition, it is known that fenofibrate increases the expression of apo A-I and apo A-II that are major construction proteins in HDL to accelerate the HDL production in the liver[15]. Actions were also reported such as, decreases in plasma levels of Lp(a)[16], uric acid[17], fibrinogen[18], and PAI-I[19]. Anti-inflammation activity in the blood vessel wall is recently reported as activity of fenofibrate[20].

The Diabetes Atherosclerosis Intervention Study (DAIS) was implemented as large-scale clinical trial in which fenofibrate was utilized; when diabetics who complicated hyperlipemia were administered with fenofibrate for 3 years or longer, TC and TG respectively decreased about 10% and about 20%, and HDL-C increased about 7% in comparison with those before administration of the drug. As a result, 40% depression of progression of coronary arteriosclerosis is verified by the quantitative analysis for the coronary artery by contrast medium[5].

Total 13 diabetics with hyperlipemia who were outpatients of our hospital were enrolled in the present study. The patients were administered with fenofibrate at daily doses of 200 mg or 300 mg for 6 months to examine the clinical effects of this drug. The results indicated that fenofibrate significantly decreased the concentration of TC and TG in the serum. In addition, we examined plasma levels of RLP-C and RLR-TG, as well as mid-band as index of remnant lipoprotein. Remnant lipoprotein is retarded intermediate metabolites of TG-rich lipoprotein. Since chyromicron and VLDL are macromolecules, they cannot invade as such into the cells composing the blood vessel wall[21], whereas the remnant lipoprotein can be taken into the blood vessel wall to cause accumulation of atheroma. Through this accumulation, the remnant lipoprotein is thought to be atherogenic lipoprotein. In type 2 diabetics who show the increase in generation of VLDL and the decrease in LPL activity, increase in the amounts of remnant lipoprotein is reported[22]. In the present study, both RLP-C and RLP-TG were significantly decreased within 3 months after the initiation of administration of fenofibrate, and the low levels persisted even in 6 months after administraiton. In 3 months after administration, RLP-C markedly decreased although the decrease in the serum TC was small; the results indicating that fenofibrate was likely to possess the activity to improve the metabolism of the remnant lipoprotein prior to the improvement of LDL metabolism. In 8 of 13 cases, the mid-band was observed to disappear on PAGE. The LDL particle sizes simultaneously determined in the present study were compared before and after administration of fenofibrate: probable enlargement of LDL particle size was indicated, because LDL-MI was significantly decreased in 6 months after administration of fenofibrate. In hypertriglyceridemia in diabetes, the small, dense LDL is known to emerge with high frequency[3]. Since the small, dense LDL gives low affinity to LDL receptor(s) and is facile to be subjected to oxidative degeneration[23, 24], the small, dense LDL is known to intensively cause atherosclerosis together with the remnant lipoprotein. A possible mechanism of fenofibrate is thought to be that this drug increases the LDL particle size via improvement of hypertriglyceridemia.

From these effects on the remnant lipoproteins and LDL particle size, we confirmed that fenofibrate is recommendable as suitable drug in the treatment of type 2 diabetes with hyperlipemia. Double blind comparison study

with placebo group is still more required, in order to confirm the result of this small clinical experiment.

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