Comparison of effects of bezafibrate and fenofibrate on circulating proprotein convertase subtilisin/kexin type 9 and adipocytokine levels in dyslipidemic subjects with impaired glucose tolerance or type 2 diabetes mellitus: Results from a crossover study

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Abstract

Background: Bezafibrate and fenofibrate show different binding properties against peroxisome proliferator-activated receptor subtypes, which could cause different clinical effects on circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and on various metabolic markers. **Methods:** An open, randomized, four-phased crossover study using 400mg of bezafibrate or 200mg of fenofibrate was performed. Study subjects were 14 dyslipidemia with impaired glucose tolerance or type 2 diabetes mellitus (61 ± 16 years, body mass index (BMI) 26 ± 3 kg/m^2, total cholesterol (TC) 219 ± 53 mg/dL, triglyceride (TG) 183 ± 83 mg/dL, high-density lipoprotein-cholesterol (HDL-C) 46 ± 8 mg/dL, fasting plasma glucose 133 ± 31 mg/dL and HbA1c 6.2 ± 0.8%). Subjects were given either bezafibrate or fenofibrate for 8 weeks, discontinued for 4 weeks and then switched to the other fibrate for 8 weeks. Circulating PCSK9 levels and other metabolic parameters, including adiponectin, leptin and urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured at 0, 8, 12 and 20 weeks. **Results:** Plasma PCSK9 concentrations were significantly increased (+39.7% for bezafibrate and +66.8% for fenofibrate, $p < 0.001$) in all patients except for one subject when treated with bezafibrate. Both bezafibrate and fenofibrate caused reductions in TG (-38.3%, $p < 0.001$ *vs.* -32.9%, $p < 0.01$) and increases in HDL-C (+18.0%, $p < 0.001$ *vs.* +11.7%, $p < 0.001$). Fenofibrate significantly reduced serum cholesterol levels (TC, -11.2%, $p < 0.01$; non-HDL-C, -17.3%, $p < 0.01$; apolipoprotein B, -15.1%, $p < 0.01$), whereas bezafibrate significantly improved glucose tolerance (insulin, -17.0%, $p < 0.05$) and metabolic markers (γ-GTP, -38.9% $p < 0.01$; adiponectin, +15.4%, $p < 0.05$; urine 8-OHdG/Cre, -9.5%, $p < 0.05$). **Conclusion:** Both bezafibrate and fenofibrate increased plasma PCSK9 concentrations. The addition of a PCSK9 inhibitor to each fibrate therapy may achieve beneficial cholesterol lowering along with desirable effects of respective fibrates.

Introduction

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a member of the subtilisin-like serine convertase superfamily made and secreted by the liver into the plasma. Secreted PCSK9 regulates plasma LDL-cholesterol (LDL-C) levels by directing cell-surface LDL receptors (LDLR) to the lysosomes for degradation, resulting in reduced clearance and accumulation of LDL-C in the circulation [1-3]. Statins have been known to increase the nuclear translocation of sterol-regulatory element binding protein-2 (SREBP-2), which activates not only the LDLR but also PCSK9 gene expression [4-6]. Subsequently, statins increases serum PCSK9 levels [5-8] and this increment may attenuate the LDL-C lowering effect of statins. It may in a part explain the rule of 6% for statins, which indicates that each doubling of the statin dose results in only about a 6% further decrease in LDL-C.

Unlike statin treatment, the association of fibrate treatrment with PCSK9 is less clear. Recently, it was shown that fenofibrate suppressed PCSK9 expression at the promoter level and reduced statin-induced PCSK9 secretion in vitro [9], whereas, fenofibrate did not suppress and rather increased circulating PCSK9 level in several in vivo studies [8, 10, 11]. Bezafibrate is another widely used fibrate, and both of which have substantial triglyceride (TG)-reducing-effect and high-density lipoprotein cholesterol (HDL-C)-raising effect. Bezafibrate is a pan-agonist for peroxisome proliferator-activated receptor (PPAR)-α, β and δ and fenofibrate is a more selective ligand for PPAR-α [12], and thus the effects of these drugs varies in several aspects. To the best of our knowledge, there is no previous study on the effect of bezafibrate treatment on PCSK9.

In this background, we directly compared the effect of fenofibrate and bezafibrate treatment on plasma PCSK9 and other metabolic parameters related to insulin sensitivity in crossover design.

2. Materials and Methods

2.1. Study subjects

To assess the effects of bezafibrate and fenofibrate on plasma PCSK9, glucose tolerance and lipid metabolism, we enrolled 14 dyslipidemic subjects with impaired glucose tolerance or type 2 diabetes mellitus (T2DM); 11 men and 3 women, age 60.5 ± 15.6 years, body mass index (BMI) 26.0 ± 3.1 kg/m^2, total cholesterol (TC) 219.0 ± 52.7 mg/dL, TG 183.0 ± 82.9 mg/dL, HDL-C 45.9 ± 8.4 mg/dL, fasting plasma glucose 133.0 ± 30.8 mg/dL and HbA1c 6.22 ± 0.85%. Among them, 5 were on anti-diabetic drug and 7 were on anti-hypertensive drug, and the dosages of these treatments were not changed during the study period. None of the subjects were on insulin treatment or anti-lipid drugs other than fibrates.

Exclusion included: age > 75 years, BMI > 30 kg/m^2, HbA1c > 7.5%, serum TG level > 400mg/dL, abnormal liver or muscle enzymes, creatinemia, habitual alcohol intake > 3 standard drink/day or endocrinological disorder.

2.2. Study protocol

This study was conducted as open randomized crossover design to compare the efficacy and the safety of bezafibrate and fenofibrate. All the participants were outpatients and were divided into two groups by envelope-method (Table 1). The group 1 (6 male, average age of 63.0 years) was started with 400 mg/day of bezafibrate, while the group 2 (5 male and 3 female, average age of 58.6 years) with 200mg/day of fenofibrate. Any lipid lowering agents had been discontinued at least for 4 weeks before the study. The first phase was continued for 8 weeks, then bezafibrate or fenofibrate were discontinued for 4 weeks, and then subsequently patients were switched to the other fibrate treatment and continued it for another 8 weeks.

A statement of institutional approval of the study in accordance with the Declaration of Helsinki

was provided, and written informed consent was obtained from all of the participants in this study.

2.3. Measurement of the laboratory data

Blood samples were obtained after an overnight fasting, and centrifuged at 4 °C. Serum TC and TG were determined by enzymatic method, and HDL-C levels were measured by a polyamine-polymer/detergent method (Daiichi Pure Chemical, Tokyo, Japan) as described elsewhere [13]. LDL-C levels were calculated using the Friedewald formula. Apolipoprotein A1, A2, B, C2, C3 and E were determined as described previously [14]. Plasma PCSK9 concentrations were determined using a commercially available quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit targeting human PCSK9 following the manufacturer instructions (Circulex CY-8079, CycLex Co, Nagano, Japan). The plasma levels of adiponectin and leptin were determined by the previously reported methods [15, 16]. The levels of Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) were determined using an ELISA kit (Stressgen, British Columbia, Canada) in accordance with the manufacturer's instructions. Other laboratory values**,** including fasting plasma glucose, HbA1c, glycoalbumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (γ-GTP) were obtained using commercially available kits with an autoanalyzer. Serum insulin levels were determined by enzyme immunoassay. Uric Acid was determined using the uricase method. Homeostasis model assessment-Insulin Resistance (HOMA-IR) was calculated using the formula: HOMA-IR = (fasting insulin in mU/l × fasting plasma glucose in mg/dl)/405.

2.4. Statistics

All values in the text and tables are expressed as mean ± SD unless otherwise stated. Effects of each drug therapy on each variable were analyzed by means of paired *t*-test. Treatment effects were

compared between two fibrates using a mixed model ANOVA. Linear correlations were analyzed using Pearson's correlation coefficient analysis. All statistical analyses were performed with PASW statistics 17.0.3 (SPSS, Chicago, Illinois). A *p*-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Characteristics of study subjects at baseline were similar in both groups

In either group 1 or 2, parameters related to lipid, glycemic control and other markers did not significantly differ before starting either fibrate. For further analysis, the data from both groups were combined and analyzed in detail. Pre- and post-treatment values of each parameter were shown in Table 2. There were no variables that would indicate carry-over and time-dependent effects.

3.2. Bezafbrate decreased γ-GTP, whereas fenofibrate showed better effect on uric acid than bezafibrate

There was no significant change in BMI and AST between values before and after treatment. There was a slight but not significant reduction in ALT during either treatment. γ-GTP decreased with bezafibrate ($p < 0.01$), but not with fenofibrate. Uric acid decreased with fenofibrate ($p < 0.001$), whereas slightly increased with bezafibrate ($p < 0.05$), and the effects of two fibrates on uric acid were significantly different from each other ($p < 0.001$).

3.3. Fenofibrate had better effects on cholesterol metabolism than bezafibrate, though both fibrates increased plasma PCSK9 levels

Plasma PCSK9 levels were increased in 13 of 14 subjects with bezafibrate (+39.7%, $p < 0.001$, Figure 1A) and in all subjects with fenofibrate (+66.8%, $p < 0.001$, Figure 1B).

Both bezafibrate and fenofibrate treatments were associated with reductions in serum TG (bezafibrate, $p < 0.001$; fenofibrate $p < 0.01$), and increases in HDL-C ($p < 0.001$ for either of treatment). There were significant reductions in TC ($p < 0.01$), apolipoprotein B ($p < 0.01$) and non-HDL-C ($p < 0.01$), and slight decrease in LDL-C during fenofibrate treatment, whereas bezafibrate did not produce reductions in these parameters. As a result, cholesterol lowering effect of fenofibrate were significantly stronger than that of bezafibrate ($p < 0.05$ for TC, LDL-C and apolipoprotein B).

Serum levels of apolipoprotein A1 and A2 were significantly increased ($p < 0.001$) and those of apolipoprotein C3 were significantly decreased ($p < 0.01$) during both of these treatments. Apolipoprotein E level was slightly but significantly decreased during bezafibrate treatment ($p < 0.05$). There were no significant changes in apolipoprotein C2 levels between values before and after fenofibrate or bezafibrate treatments.

3.4. Bezafibrate had better effects on glucose tolerance than fenofibrate

Changes in plasma glucose and glycoalbumin were not significant during either treatment. Fenofibrate was associated with a slight but significant increase in HbA1c ($p < 0.05$) whereas bezafibrate was not. Insulin level and HOMA-IR significantly decreased with bezafibrate ($p < 0.05$) but not with fenofibrate.

3.5. Bezafibrate improved serum adiponectin level and oxidative stress marker

Bezafibrate but not fenofibrate was associated with a significant increase in serum adiponectin ($p < 0.05$) and with a significant decrease in urine 8-OHdG/Cre ($p < 0.05$). Serum leptin did not significantly alter in either treatment.

3.6. The associations of plasma PCSK9 with cholesterol and several metabolic markers

As shown in Table 3, circulating PCSK9 levels were significantly correlated with TC ($r = 0.477$, $p = 0.01$), LDL-C ($r = 0.501$, $p < 0.01$), non-HDL-C ($r = 0.453$, $p < 0.05$), BMI ($r = 0.377$, $p < 0.05$), γGTP ($r = 0.376$, $p < 0.05$), plasma glucose ($r = -0.426$, $p < 0.05$), HbA1c ($r = -0.468$, $p < 0.05$), glycoalbumin ($r = -0.563$, $p < 0.01$), insulin ($r = 0.507$, $p < 0.01$), HOMA-IR ($r = 0.396$, $p < 0.05$) and leptin ($r = 0.416$, $p < 0.05$) at baseline levels. When analyzed using absolute or percent change value for each variable during the treatment, no significant associations were observed in the present study.

4. **Discussion**

The main finding of the present study is that all subjects with fenofibrate treatment and all but one subjects with bezafibrate treatment showed increases in their circulating PCSK9 levels. The degree of increase in PCSK9 tended to be higher in fenofibrate treatment than in bezafibrate treatment (+66.8% *vs.* +39.7%, $p = 0.077$). This is the first study investigating the differences between bezafibrate and fenofibrate in their effects on circulating PCSK9 level and other metabolic parameters related to insulin resistance and oxidative stress in Japanese hyperlipidemic subjects.

As observed in non-diabetic patients [17-19], we found that plasma PCSK9 levels positively correlated with TC, LDL-C, non-HDL-C, BMI, γGTP, insulin and HOMA-IR at baseline levels in subjects with impaired glucose tolerance or T2DM. On the other hand, fasting plasma glucose was negatively correlated with plasma PCSK9 levels in our study on impaired glucose tolerance or type 2 diabetes mellitus, which does not appear to be consistent with these reports on non-diabetic patients. Very recently, however, it was shown that PCSK9 knockout mice had higher glucose and lower insulin levels than wild-type mice [20], suggesting some association of PCSK9 with increasing insulin leading to lowering plasma glucose. This may in part explain the negative correlation of

PCSK9 with plasma glucose and HbA1c in our study.

Bezafibrate and fenofibrate have some evidence of preventing coronary artery disease in Bezafibrate Infarction Prevention (BIP) [21, 22] and Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study [23], respectively. Our present data indicate that fenofibrate treatment (200 mg per day for 8 weeks) produced more favorable effects on cholesterol metabolism than did bezafibrate treatment (400 mg per day for 8 weeks) with regard to lower TC, non-HDL-C, LDL-C and apolipoprotein B levels. Circulating PCSK9 protein levels were significantly increased by either of these two fibrates,

Previous reports showed conflicting findings on the effect of fenofibrate on PCSK9. Kourimate et al. demonstrated that PPAR-α activation resulted in the repression of PCSK9 promoter activity, and in the up-regulation of PC5/6A and furin, which degrade PCSK9 [9]. To our knowledge, two clinical studies suggest that fenofibrate treatment was associated with decrease in PCSK9 levels [24, 25]. Lambert et al. [24] reported that fenofibrate 200 mg/day was associated with decrease (-8.5%; $p = 0.041$) in PCSK9 level in diabetic patients and Chan et al. [25] showed fenofibrate 145 mg/day was associated with decrease (-13%; $p < 0.05$) in statin treated T2DM subjects. In contrast to these reports, Mayne et al. and Troutt et al. represented an increase in circulating PCSK9 level after fenofibrate treatment with 200 mg/day in dyslipidemic patients (+17%; $p = 0.031$ and +25%; $p < 0.01$, respectively) [8, 10], although the precise mechanism contributing to these increases remains to be clarified. Furthermore, Costet et al. reported that 6 weeks of treatment with either 10 mg/day atorvastatin or 160 mg/day fenofibrate increased PCSK9 levels (+14%; $p = 0.01$ and +26%; $p < 0.01$, respectively) in T2DM subjects, with no additive effect after 6 weeks of combined therapy [11]. In our study, the effect of fenofibrate treatment with 200 mg/day on plasma PCSK9 level was more prominent (+66.8%) than these reports and was even higher than previously reported value of high dose (80 mg) atorvastatin treatment (+47%) [26], although study population and duration of the

treatment were different.

In patients with T2DM, insulin resistance inhibits catabolism of remnant lipoproteins by reducing lipoprotein lipase (LPL) activity, contributing to the development of hypertriglyceridemia and low HDL-C [27-29]. The present data indicate that bezafibrate and fenofibrate had equivalent efficacy on TG and HDL-C improvement in subjects with hyperglycemia. As a PPAR-α agonists, these fibrates improve triglyceride and HDL-C concentrations by enhancing β-oxidation of fatty acids and LPL activity, increasing production of the components of HDL (apolipoproteins A1 and A2), and reducing production of the inhibitor of LPL activity (apolipoprotein C3) [30-34], while enhancing cholesterol efflux from the liver [35]. Several reports [8, 10] suggest that these effects of PPAR-α on cholesterol and lipoprotein metabolism may lead indirectly to decrease hepatic intracellular cholesterol levels, and thus result in a secondary increase in PCSK9 expression and secretion. Costet et al. reported that one-day administration of atorvastatin increased PCSK9 levels whereas that of fenofibrate did not [11], which might support the indirect slower effect of PPAR-α agonist on PCSK9 expression.

PPAR-γ is another target of bezafibrate and is expected to have effects on glucose metabolism and insulin resistance [36]. Several previous studies have indicated that bezafibrate treatment could be associated with improvement of glycemic control in diabetic subjects [37, 38] or with delay of the onset of T2DM [39, 40]. As for fenofibrate treatment, on the other hand, there are conflicting reports on its effect on glycemic control [41, 42]. In our previous study [43], fasting plasma glucose level was unchanged after 8 weeks of treatment with fenofibrate, which is consistent with the present study. Of note, the very recent retrospective cohort study using data from routine medical practice in the United Kingdom [44] has shown that compared to fenofibrate, bezafibrate was associated with a particularly low hazard for the occurrence of T2DM (HR 0.41, 95% CI 0.29, 0.58). Indeed, our present findings suggest that bezafibrate is more desirable than fenofibrate concerning serum insulin level and HbA1c.

The mechanism by which the degree of PCSK9 increases was more modest in bezafibrate than in fenofibrate is not clarified yet. Costet et al. reported that insulin increased hepatic PCSK9 mRNA expression in rodent primary hepatocytes and in vivo during a hyperinsulinemic-euglycemic clamp in mice [45]. This finding combined with our present result that insulin level positively correlated with plasma PCSK9 level at baseline and that insulin level was decreased with bezafibrate but not with fenofibrate may in part explain the more modest increase in PCSK9 levels produced by the former than the latter.

In addition to the effect on glycemic control, these fibrates may have beneficial effects on adipocytokines, such as leptin, which positively correlated with PCSK9 in the present study, and adiponectin [41, 46-49]. Damci et al. showed a reduction in leptin levels with fenofibrate treatment (250 mg/day for 3 months) [41], whereas no significant change in leptin levels was observed in either fibrate treatment in the present study with a shorter duration. For the effect of fibrate treatment on serum adiponectin, the present finding that fenofibrate did not increase serum adiponectin is consistent with our previous study [43], although some studies have shown associations of fenofibrate treatment with adiponectin increase in humans [46, 47]. On the other hand, bezafibrate treatment was associated with an increase in serum adiponectin in the present study. Indeed, there are several previous studies linking bezafibrate to an increase in serum adiponectin in patients enrolled in the BIP study [48] and in spontaneous T2DM model (Otsuka Long-Evans Tokushima Fatty; OLETF) rats [49]. Hiuge et al. also reported that bezafibrate and fenofibrate significantly increased adiponectin levels in mice and 3T3-L1 adipocytes [48].

For the association between fibrate and liver function, there is a report linking bezafibrate treatment to an improvement of γ-GTP in subjects with chronic liver disease [50], which is compatible with the present finding although subjects were not chronic liver disease in our study. Consistent with the report by Cariou et al. [18], γ-GTP positively correlated with PCSK9 at baseline in our study, and

thus the significant decrease in γ-GTP only with bezafibrate might be related to the milder increase in circulating PCSK9 levels with bezafibrate than fenofibrate. A significant reduction in uric acid levels by fenofibrate in the present study is consistent with previous report [51], whereas there is no previous report on the effect of bezafibrate treatment on uric acid.

Angiopathy of T2DM has a complex etiology that may involve the effects of hyperlipidemia and oxidative stress on endothelial function. Similar to certain types of lipid lowering agents such as probucol and atorvastatin [52], we observed beneficial effect of bezafibrate to produce significant decreases in urine 8-OHdG/Cre, which has been recognized as an important oxidative stress marker.

One of the limitations of our study is the small sample size. However, the study was accomplished in the cross over design, making it possible to investigate the effect of bezafibrate and fenofibrate treatments in the same subjects and thereby enabling to exclude the possibility of study subject bias. Moreover, the results were clear and convincing especially in PCSK9 changes during fibrate treatment.

In conclusion, both bezafibrate and fenofibrate cause considerable increase in plasma PCSK9 in hyperlipidemic subjects with the former in more modest manner. We suggest that in the treatment of hyperlipidemic subjects, the addition of a PCSK9 inhibitor to each fibrate therapy may achieve beneficial cholesterol lowering along with desirable effects of respective fibrates.

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Disclosure

The contents of this paper have not been published or communicated elsewhere. The authors have no relationships with companies regarding a financial interest in the information contained in this paper.

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Figure legend

Figure 1. Changes in circulating PCSK9 concentrations in response to 8 weeks of treatment with (A) 400 mg of bezafibrate per day or (B) 200 mg of fenofibrate per day. Open circles represent means of 14 subjects and bars S.D. *** $p < 0.001$ *vs*. pre-treatment value.