Effect of Bezafibrate or Pravastatin on Serum Lipid Levels and Albuminuria in NIDDM Patients

Takashi Nagai¹, Takashi Tomizawa¹, Katsuyuki Nakajima², and Masatomo Mori³

¹Department of Internal Medicine, Public Tomioka General Hospital, Gunma, Japan.
²Japan Immunoresearch Laboratories, Gunma, Japan.
³First Department of Internal Medicine, Gunma University School of Medicine, Gunma, Japan.

Lipid abnormalities in diabetic patients, particularly in those with nephropathy, may be partially due to deteriorating atherosclerosis. Therefore, strict control of the lipid metabolism in addition to glycemic control is desirable. Whether or not lipid lowering drugs prevent albuminuria in diabetic patients in the long term remains unclear. This study involved 71 NIDDM patients with hypercholesterolemia (group A: n = 37, group B: N = 34). The effect of bezafibrate (group A) or pravastatin (group B) on the cholesterol (CH) content of apolipoprotein Al, B100 containing particles or remnant-like particles (RLP) or urinary albumin excretion was studied over 4 years. The CH content in apolipoprotein B100 particles after treatment with either bezafibrate or pravastatin decreased significantly (group A: 24.7%, group B: 26.6%). The CH content in RLP after treatment with bezafibrate showed a significant decrease (67.9%). Apolipoprotein Al after treatment with bezafibrate showed a significant increase (10.9%). Apolipoprotein B100 after treatment with either drug decreased significantly (group A: 19.8%, group B: 23.4%). The urinary albumin excretion rate after treatment with either drug showed no significant change over 4 years. Bezafibrate and pravastatin appear to be useful in the preventive treatment of albuminuria as well as in lowering lipid levels in NIDDM patients. J Atheroscler Thromb, 2000; 7: 91-96.

Key words: Apolipoprotein Al, B100 particles, RLP-cholesterol, Ischemic heart disease, Urinary albumin excretion

Introduction

Hypertriglyceridemia and hypercholesterolemia are frequently found in diabetic patients, particularly those with nephropathy (1). Both are thought to play some role in the high incidence of atherosclerosis in these patients. A similarity in pathogenesis between glomerular lesions due to hyperlipidemia and atherosclerotic lesions has been suggested (2). Strict control of hyperlipidemia in addition to glycemic control is important for atherosclerosis prevention in diabetic patients. Although decreased albuminuria by lipid lowering drugs in hyperlipemic diabetic patients over 3 months has been reported (3), it remains unclear whether or not lipid lowering drugs can be successful in preventing albuminuria in diabetic patients over a long period.

We analyzed apolipoprotein Al, B100 containing particles or remnant-like particles (RLP) reflecting chylomicron or VLDL remnants which are most likely to be atherogenic particles (4). These have been isolated by immunoaffinity columns prepared with monoclonal antibodies in NIDDM patients with hypercholesterolemia. We also investigated the effects of pravastatin, a 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor class of CH-lowering drug (5) or bezafibrate, a leading member of the fibrate family which regulates both the cholesterol (CH) and triglyceride metabolisms (6). We examined the CH content of these particles and urinary albumin excretion in NIDDM patients with hypercholesterolemia over a four-year-period.
Subjects

This study involved 71 age-matched NIDDM men with hypercholesterolemia (fasting total CH > 5.7 mmol/l). The patients were randomly divided into two groups (group A + B). Among the 37 patients in group A were 13 with diabetic retinopathy. In group B, of 34 patients there were 11 with diabetic retinopathy. All body mass indices (BMI) were less than 25 kg/m² and all urinary specimens showed to± proteinuria and negative for hematuria or urinary cast. All diabetic patients showed a normal range of hematology, liver function and electrolytes. Their double two-step exercise test was within normal limits. All diabetic patients were on a diabetic diet including protein of 1.0 to 1.2 g/kg body weight. Twenty-five patients (group A : n = 13, group B : n = 12) were given an oral hypoglycemic drug (sulfonylurea). In 33 patients (group A : n = 17, group B : n = 16), insulin treatment (mean administration of insulin dose : group A : 18.9±2.3 units, group B : 19.2±2.1 units) was given for control of blood glucose. All diabetic patients had been treated for a minimum of 6 months and their blood glucose, total CH or triglyceride had remained stable for at least 3 months. Their HbA1c was less than 7.9%. Anti-hypertensive drugs were given in group A (n = 22, sixteen patients were given angiotensin converting enzyme inhibitor, cilazapril [Eizai Co. Ltd., Tokyo] 1 mg daily, of the 6 patients were given 2 mg daily.) and group B (n = 21, fifteen patients were given cilazapril 1mg daily, of the 6 patients were given 2 mg daily.). Those patients had been treated blood pressure for a minimum of 6 months. Their systolic blood pressure was less than 150 mmHg and their diastolic blood pressure was less than 90 mmHg over a period of 3 months or more in each group.

Methods

Measurement of total CH content in apolipoprotein Al particles (Apo Al-CH), apolipoprotein B100 particles (Apo B100-CH) or remnant-like particles containing CH values (RLP-CH) was as follows:

Five mg of anti-apolipoprotein Al (H-12), anti-apolipo-
protein B100 (Jl-H) or anti-apolipoprotein Al and anti-
apolipoprotein B100 monoclonal antibodies were coupled to 1 ml of CNBr-Sepharose 4B affinity column (Pharmacia, Sweden) (4) according to the recommendations of the manufacturer. Then the remaining active sites on the columns were blocked with 0.2 M glycine. Before use, the columns were conditioned to reduce non-specific binding by 2 cycles of incubation with fetal calf serum for 1 hr at room temperature with gentle shaking, followed by washing with 0.1 M acetic acid in 0.5 M NaCl. Five µl of serum was added to 300 µl of immunoaffinity column suspension which contained 25 µl of apolipoprotein Al (125 µg of IgG), 25 µl apolipoprotein B100 (125 µg of IgG), or 25 µl of apolipoprotein Al and apolipoprotein B100.

The reaction mixture was gently shaken for 60 min. at room temperature to assure complete mixing. The bound fraction of Apo Al-CH and Apo B100-CH, and the unbound fraction of RLP-CH were eluted from the mixed gels with 1.0 M acetic acid. The fraction tubes were allowed to sit for 10 min. and 30 µl of the supernatant was taken to assay Apo Al-CH, Apo B100-CH and RLP-CH with the Mercos test diagnostic kit (Kanto-kagaku, Tokyo). The coefficient of variation of Apo Al-CH or Apo B100-CH or RLP-CH measurements was less than 2.5%.

Apolipoprotein Al and B100 values were measured by turbidimetric immunoassay. Triglyceride values were measured by enzymatic determination. LDL-CH was measured by precipitation and enzymatic determination. HDL-CH was calculated according to Friedewald et al. (7).

Insulin was measured by a radioimmunoassay. FIRI was calculated as fasting blood glucose (mmol/l) × basal insulin (µU/ml) / 25 (8).

Renal function

Creatinine (Cr) was measured by Jaffe’s rate assay. Urinary samples were collected from the first morning urine excretion. Urinary albumin was measured by radioimmunoassay. The urinary albumin excretion rate (Alb-l) was calculated as urinary albumin/urinary Cr (mg/ gCr).

Table 1. Backgroud data of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>group A</th>
<th>group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>37</td>
<td>34</td>
</tr>
<tr>
<td>age (years)</td>
<td>60.6±2.1</td>
<td>60.2±2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8±0.2</td>
<td>22.7±0.4</td>
</tr>
<tr>
<td>systolic BP (mmHg)</td>
<td>141.5±2.6</td>
<td>142.2±2.2</td>
</tr>
<tr>
<td>diastolic BP (mmHg)</td>
<td>81.8±2.2</td>
<td>82.1±2.2</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>6.39±0.28</td>
<td>6.36±0.31</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.2±0.2</td>
<td>7.1±0.2</td>
</tr>
<tr>
<td>basal insulin (µU/ml)</td>
<td>8.9±0.4</td>
<td>8.8±0.3</td>
</tr>
<tr>
<td>FIRI (FBG×basal insulin/25)</td>
<td>2.28±0.21</td>
<td>2.24±0.2</td>
</tr>
<tr>
<td>creatinine (µmol/l)</td>
<td>79.8±7.5</td>
<td>81.2±7.3</td>
</tr>
<tr>
<td>Alb-I (mg/g × creatinine)</td>
<td>107.5±16.4</td>
<td>102.2±20.2</td>
</tr>
<tr>
<td>triglyceride (mmol/l)</td>
<td>1.81±0.11</td>
<td>1.81±0.15</td>
</tr>
<tr>
<td>total-CH (mmol/l)</td>
<td>6.48±0.19</td>
<td>6.49±0.19</td>
</tr>
<tr>
<td>HDL-CH (mmol/l)</td>
<td>1.26±0.06</td>
<td>1.22±0.06</td>
</tr>
<tr>
<td>ApoAI-CH (mmol/l)</td>
<td>1.34±0.06</td>
<td>1.29±0.06</td>
</tr>
<tr>
<td>Apolipoprotein Al (mg/dl)</td>
<td>131.5±4.4</td>
<td>130.2±3.5</td>
</tr>
<tr>
<td>LDL-CH (mmol/l)</td>
<td>4.39±0.27</td>
<td>4.53±0.28</td>
</tr>
<tr>
<td>ApoB100-CH (mmol/l)</td>
<td>4.97±0.27</td>
<td>4.94±0.26</td>
</tr>
<tr>
<td>Apolipoprotein B100 (mg/dl)</td>
<td>121.6±4.0</td>
<td>123.4±4.1</td>
</tr>
<tr>
<td>RLP-CH (mmol/l)</td>
<td>0.24±0.04</td>
<td>0.25±0.04</td>
</tr>
</tbody>
</table>

group A : diabetic group with bezafibrate administration.
group B : diabetic group with pravastatin administration.
Lipid Lowering and Albuminuria

Treatment
NIDDM patients accompanied with hypercholesterolemia were orally given either 400 mg of bezafibrate (Kissei Co. Ltd., Tokyo) (group A) or 10 mg of pravastatin (Sankyo Co. Ltd., Tokyo) (group B) daily for 48 months.

Statistical analysis
Parameters are shown as mean±SEM. The data were analyzed by Duncan’s multiple range test. Correlation between Apo AI-CH and HDL-CH or Apo B100-CH and LDL-CH in all subjects was determined by a linear regression analysis. A level of p<0.05 was accepted as statistically significant.

Results
As shown in Table 1, BMI, systolic blood pressure, diastolic blood pressure, fasting blood glucose, HbA1c, basal insulin, FIRI, Cr, Alb-1, triglyceride, total-CH, HDL-CH, LDL-CH, Apo AI-CH, Apo B100-CH, RLP-CH, apolipoprotein Al and B100 levels were not significant different before administration of the study between group A and group B. While the correlation between Apo AI-CH and HDL-CH was 0.596 (p<0.01), the correlation between Apo B100-CH and LDL-CH was 0.812 (p<0.01) in all subjects.

As shown in Fig. 1, fasting blood glucose, HbA1c and BMI had no significance over 4 years in each group (mean HbA1c : 7.1±0.2% in group A, 7.1±0.2% in group B). FIRI remained unchanged in group B. A decrease was
noted in group A, but it was not significant.

As shown in Fig. 2, significant reductions in triglyceride (44.6%), total-CH (18.9%) and Apo B100-CH (24.7%) occurred after administration of bezafibrate over 4 years. An increase in Apo Al-CH (11.0%) took place after administration of the drug. However, it was not significant. Significant reductions in total-CH (20.3%) and Apo B100-CH (26.6%) occurred after administration of pravastatin over 4 years.

As shown in Fig. 3, a significant increase in apolipoprotein Al (10.9%) and significant decreases in RLP-CH (67.9%) and apolipoprotein B100 (19.8%) were also noted after administration of bezafibrate over a four-year period. A significant decrease in apolipoprotein B100 (23.4%) occurred after administration of pravastatin over 4 years.

As shown in Fig. 4, systolic blood pressure, diastolic blood pressure, creatinine and urinary albumin excretion remained almost unchanged over 4 years in each group (mean Alb-I : 102.6±22.8 mg/gCr (12 months), 109.2±23.8 mg/gCr (48 months) in group A, 104.8±24.4 mg/gCr (12 months), 107.2±24.7 mg/gCr (48 months) in group B). Systolic blood pressure ranged less than 150 mmHg and diastolic blood pressure ranged less than 90 mmHg in each group over 4 years (mean systolic blood pressure : 141.9±2.6 mmHg in group A, 142.2±2.5 mmHg in group B, mean diastolic blood pressure : 82.3±2.1 mmHg in group A, 82.3±2.1 mmHg in group B).

No abnormalities were noted in general laboratory findings including hematology and chemistry during the 4-year treatment. The mean dose of insulin remained nearly unchanged (group A : 19.4±2.6 units, group B :
Lipid Lowering and Albuminuria

19.7 ± 2.7 units). During the four-year-period of the study, diabetic retinopathy occurred in 2 patients, one from group A and one from group B. Over the same period it also disappeared in one patient from group A and one from group B. It remained unchanged in other patients over the course of this study. The double two-step exercise test induced ischemic ST-segment depression and the coronary angiogram showed stenosis (75%), leading to diagnosis of ischemic heart disease in one patient from group A and one patient from group B after 4 years, although the test showed a normal limit within the first 3 years.

Discussion

Many of our NIDDM patients take anti-hypertensive drugs (59–62%), however, cilazapril has no significant effect on body weight, plasma glucose or insulin levels (9). Total-CH levels in the 2 groups decreased significantly during the 48 months of treatment, but during this period, fasting blood glucose, HbA1c and BMI levels were virtually unchanged in both groups. Therefore, the decrease of total-CH levels after administration of each drug was independent of diabetic metabolic abnormality. The correlation between Apo AI-CH and HDL-CH as well as Apo B100-CH and LDL-CH was significantly positive. Bezafibrate decreased Apo B100-CH and apolipoprotein B100 significantly, while it increased apolipoprotein AI significantly. There was also some Apo AI-CH increase, but it was not significant. These results are compatible with the previous reports (6, 10). The observation that pravastatin decreases Apo B100 CH and apolipoprotein B100 significantly is also compatible with previous reports (5, 11).

After treatment with bezafibrate, triglyceride and RLP-CH showed significant decreases. However, changes with pravastatin were not significant. The significant triglyceride decrease due to bezafibrate administration could be attributed to activated lipoprotein lipase and hepatic triglyceride lipase (6). The RLP-CH assay provides a measure of those particles with β or pre-β mobility by agarose electrophoresis (12) that are most likely to be atherogenic. Bezafibrate enhances conversion of VLDL to LDL and conversion of IDL to LDL. Therefore, bezafibrate is effective in reducing RLP-CH.

The decrease in FIRI, which reflects insulin resistance (8), occurred after administration of bezafibrate, however, it was not significant in our study. Moreover, HbA1c and mean administration of insulin dose remained nearly unchanged after bezafibrate administration. Since bezafibrate has been reported to both improve and also to have no effect on insulin sensitivity (13, 14), further study may be worthwhile.

Both drugs were associated with a reduction in total-CH, apolipoprotein B100 and Apo B100-CH (particularly strong with pravastatin). Bezafibrate led to a fall in triglyceride and RLP-CH and a rise in apolipoprotein AI. Ischemic heart disease was, however, noted in each group (group A: 2.7%, group B: 2.9%) after 4 years. Both drugs induced nearly the same preventative effect on atherosclerosis in NIDDM patients.

Pravastatin has been shown to decrease albuminuria in hyperlipidemic diabetic patients over a 3-month period (3). Glomerular lesions due to hyperlipidemia and atherosclerotic lesions have exhibited a similarity in pathogenesis (2). LDL stimulates mesangial fibronectin production, inducing extracellular matrix expansion (15). Pravastatin inhibits mesangial cell proliferation by means other than lipid-lowering (16). These drugs may ameliorate glomerular injury by not only reducing serum lipid levels, but also by inhibiting mesangial cell proliferation. Urinary albumin excretion did not increase in either group over 48 months. HbA1c levels in our groups were almost the same as in the good HbA1c group (using intensive treatment, which slows the development of diabetic nephropathy) in DCCT (17). However, the age and systolic and diastolic blood pressure levels in our groups were significantly higher than in the DCCT group. Although systolic blood pressure elevation accelerates the development of diabetic nephropathy (18), urinary albumin excretion did not increase in either group over 48 months. Therefore, anti-lipid drugs appear to be useful in the preventive treatment of albuminuria. In addition to controlling blood glucose and blood pressure, anti-lipid drugs may play a significant role in the prevention of deteriorated albuminuria in NIDDM patients.

References


