Ezetimibe Ameliorates Metabolic Disorders and Microalbuminuria in Patients with Hypercholesterolemia

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Aim: Ezetimibe, an inhibitor of Niemann-Pick C1-like 1 protein, has been shown to reduce the intestinal absorption of cholesterol. We investigated whether it also has beneficial effects on metabolic disorder and/or renal insufficiency in patients with hypercholesterolemia.

Methods: Ezetimibe was administered to 38 Japanese patients with hypercholesterolemia to obtain appropriate low-density lipoprotein cholesterol (LDL-chol) levels. Age- and sex-matched patients with hypercholesterolemia (n=38) were the controls. We evaluated the effects of ezetimibe before and 4 to 8 weeks after ezetimibe treatment.

Results: Ezetimibe significantly decreased LDL-chol levels and metabolic syndrome-related factors, including body weight, waist circumference, blood pressure; homeostasis model assessment insulin resistance (HOMA-IR), and urinary albumin excretion, were significantly reduced. In addition, it decreased the level of high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α, the urinary excretion of 8-hydroxy-2′-deoxyguanosine, a parameter of oxidative stress, and increased the urinary excretion of nitrate and nitrite (NOx). In the controls we observed no such changes. Excepting the decrease in the serum TNF-α level, the effects of ezetimibe were not correlated with decreased LDL-chol levels.

Conclusion: Ezetimibe ameliorated the status of metabolic syndrome and microalbuminuria, reduced inflammation and oxidative stress, and increased nitric oxide bioavailability in a LDL-chol reduction-dependent and -independent manner.


Key words; Metabolic syndrome, Chronic kidney disease, Oxidative stress, Inflammation

Introduction

There is global consensus that highly elevated low-density lipoprotein cholesterol (LDL-chol) is a major risk factor for atherosclerotic cardiovascular disease. Although statins, HMG-CoA reductase inhibitors, have been shown to effectively lower LDL-chol, classic statins fail to achieve standard treatment goals as defined by The National Cholesterol Education Program (NCEP)¹ ². Although high doses of statins or strong statins lower lipid more effectively, the risk of serious side effects appears to be dose-dependent ³ and only 67% of patients are treated successfully with high doses of statins or strong statins ⁴. Therefore, in efforts to prevent atherosclerotic cardiovascular diseases, attention has focused on the development of lipid-lowering compounds with action mechanisms different from those of statins.

Ezetimibe, a novel agent that potently inhibits
the intestinal absorption of cholesterol from dietary and biliary sources by blocking the Niemann-Pick C1-like 1 protein for cholesterol transport, is metabolized across the intestinal wall\textsuperscript{5-6}. Therefore, its effects in the liver and intestine appear to be limited and its distribution outside the intestines and/or portal system is minimal or non-existent. In addition to lipid-lowering effects, statins exert numerous cardioprotective effects by increasing the bioavailability of vascular nitric oxide (NO) and by reducing oxidative stress and inflammatory cytokines\textsuperscript{7-9}. However, it remains unknown whether ezetimibe manifests cardiorenal protective effects and improves metabolic disorders such as obesity, hypertension, insulin resistance, and renal insufficiency.

We tested our hypothesis that ezetimibe improves metabolic disorders and renal insufficiency in patients with hypercholesterolemia.

**Methods**

**Subjects**

We studied 38 patients with hypercholesterolemia whose serum lipid profiles did not reach the management goals promulgated by the new (2007) guidelines for the prevention of atherosclerotic cardiovascular diseases of the Japanese Atherosclerosis Society. The controls were 38 age- and sex-matched patients with hypercholesterolemia. The study subjects were outpatients and hospitalized patients of the Department of Cardiovascular Medicine of Tokushima University Hospital. The patient profiles are shown in Table 1; the 2 groups did not significantly differ with respect to patient characteristics. Exclusion criteria included severe left ventricular dysfunction (ejection fraction evaluated by echocardiography <40%), apparent renal disease (serum creatinine >2.0 mg/dL, urinary albumin excretion >300 mg/g creatinine), clinical conditions that could lead to increased inflammatory cytokine levels (i.e. rheumatoid arthritis and sepsis), and liver disease as defined by hepatic enzymes >2 times the upper normal limit. Also excluded were patients who were newly medicated or whose medications for hypertension, dyslipidemia, and insulin resistance had been changed less than one year before the start of this study. Prior written informed consent was obtained from all subjects before enrollment in this study in accordance with protocols approved by the Tokushima University Hospital Ethics Committee.

**Treatment**

Patients received 5 mg ezetimibe perorally daily in the morning; they continued their previous treatments for dyslipidemia, including statins and/or diet therapy. In the controls, previous treatments for dyslipidemia, excepting ezetimibe, were continued after enrollment in this study. We collected blood and urine samples before and 4 to 8 weeks (mean 4.7 ± 1.7 weeks) after the inception of this study. During the study period the patients’ medication regimens were unchanged except that they received ezetimibe, diet, and exercise therapy. Of the patients treated with ezet-

![Table 1. Patient Characteristics](https://example.com/table1.png)
imibe, 3 developed mild diarrhea; none suffered severe adverse side effects.

### Biochemical Analysis

After an overnight fast, blood samples were collected before noon from the antecubital vein. Lipid parameters, including high-density lipoprotein cholesterol (HDL-chol), triglyceride and LDL-chol, were assayed by enzymatic methods. Spot urine samples were collected and creatinine and urinary albumin were analyzed; the urinary albumin excretion ratio was calculated and expressed in mg/g creatinine. The plasma glucose level was determined with the hexokinase method; serum immunoradiometric insulin (IRI) by immunoradiometric assay. The homeostasis model assessment insulin resistance (HOMA-IR) was calculated using the formula: \[
\text{HOMA-IR} = \frac{\text{fasting serum insulin (μU/mL)}}{\text{fasting plasma glucose (mg/dL)}} / 405.
\]
Urinary excretion levels of nitrate and nitrite (NOx) as a parameter of the bioavailability of NO were measured by the Griess method (Griess reagent kit for nitrite determination; Invitrogen Japan K.K, Tokyo, Japan) and expressed in nmol/g creatinine. Tumor necrosis factor (TNF)-α was measured by ELISA (Human TNF-α TNFSF1A; R&D Systems, Inc., Minneapolis, USA), urinary excretion of 8-hydroxy-2’-deoxyguanosine (8-OHdG) as a parameter of oxidative stress was also determined by ELISA (new 8-OHdG Check ELISA Kit; Japan Institute for the Control of Aging, Nikken SEIL Corporation, Shizuoka, Japan) and expressed in μg/g creatinine. High-sensitivity C-reactive protein (hs-CRP) levels were measured at Bio Medical Laboratories (Tokyo, Japan) by nephelometry, a latex particle-enhanced immunoassay (N Latex CRP II).

### Statistical Analysis

Lipid profiles and other biomarkers were compared before and after ezetimibe treatment using the paired \( t \)-test. All data are expressed as the mean ± S.D. Single regression analysis was used to assess the correlation between the decrease in the LDL-chol level and other indices. These analyses were performed on a Microsoft Windows computer running SPSS software. Differences were considered significant at \( p < 0.05 \).

### Results

#### Effect of Ezetimibe on Lipid Profiles

Ezetimibe significantly reduced the LDL-chol level (146 ± 28.1 to 111 ± 29.3 mg/dL; −24.0%, \( p < 0.01 \)) but had no effect on triglycerides (pre- and post-treatment, 165 ± 78.8 and 149 ± 69.6 mg/dL, respectively) and HDL-chol levels (pre- and post-treatment, 55.9 ± 15.4 and 56.5 ± 16.0 mg/dL). In the controls we observed no significant changes in measurements obtained before and after the inception of this study (LDL-chol: before: 144 ± 26.5, after: 140 ± 23.3 mg/dL; triglycerides: before: 151 ± 58.4, after: 146 ± 50.4 mg/dL; HDL-chol, before: 53.9 ± 16.0, after: 54.2 ± 15.4 mg/dL) (Fig. 1).

#### Ezetimibe Treatment Resulted in a Decrease in Body Weight, Waist Circumference, and Blood Pressure

Ezetimibe treatment significantly reduced body weight (65.3 ± 12.7 to 64.2 ± 12.6 kg, \( p < 0.05 \)), body mass index (25.1 ± 6.16 to 24.3 ± 6.02 kg/m², \( p < 0.05 \)), and the waist circumference (87.6 ± 8.69 to 85.7 ± 8.51 cm, \( p < 0.05 \)). It also significantly decreased both the systolic- (139 ± 17.9 to 132 ± 15.8 mmHg, \( p < 0.05 \)) and diastolic blood pressure (75.6 ± 11.4 to 71.4 ± 9.56 mmHg, \( p < 0.05 \)), but not the heart rate.
The controls manifested no changes in measurements obtained before and after the inception of this study (body weight before: 63.5 ± 13.2, after: 63.4 ± 13.2 kg; body mass index before: 25.6 ± 6.27, after: 25.6 ± 6.33 kg/m²; waist circumference before: 86.6 ± 8.06, after: 86.2 ± 8.52 cm; systolic blood pressure before: 137 ± 18.1, after: 135 ± 18.3 mmHg; diastolic blood pressure before: 76.6 ± 12.3, after: 73.4 ± 9.90 mmHg; heart rate before: 67.6 ± 6.70, after: 68.3 ± 7.80 bpm) (Fig. 2). The effects of ezetimibe were not correlated with a decrease in the LDL-chol level.

Ezetimibe Treatment Improved Insulin Resistance and Attenuated Urinary Albumin Excretion

Ezetimibe treatment did not affect fasting plasma glucose and hemoglobin A1c levels but significantly decreased HOMA-IR as a parameter for insulin resistance (4.73 ± 3.47 to 3.83 ± 2.91, p < 0.01). In addition, it attenuated urinary albumin excretion (38.6 ± 53.6 to 26.1 ± 46.4 mg/g creatinine, p < 0.01) without changing the estimated glomerular filtration rate (61.8 ± 17.4 to 60.3 ± 17.7 mL/minute/1.73 m²). The controls manifested no changes before and after inception of the study (HOMA-IR before: 4.16 ± 3.00, after: 4.36 ± 3.09; urinary albumin excretion before: 33.5 ± 48.1, after: 30.7 ± 46.7 mg/g creatinine; estimated glomerular filtration rate before: 63.6 ± 18.4, after: 61.8 ± 18.3 mL/minute/1.73 m²) (Fig. 3). The effects of ezetimibe on HOMA-IR and urinary albumin excretion were not correlated with a decrease in the LDL-chol level.

Ezetimibe Treatment Attenuated Inflammation and Oxidative Stress and Enhanced NO Bioavailability

Ezetimibe significantly reduced the hs-CRP levels (165 ± 168 to 106 ± 138 µg/dL, p < 0.01), TNF-α levels (23.6 ± 9.33 to 16.1 ± 8.16 pg/mL, p < 0.01), and the urinary excretion of 8-OHdG (6.16 ± 4.40 to 5.53 ± 4.00 µg/g creatinine, p < 0.05). Moreover, it significantly increased the urinary excretion of NOx (18.8 ± 10.8 to 25.6 ± 18.3 nmol/g creatinine, p < 0.05). The controls exhibited no changes before and after the inception of the study (hs-CRP before: 184 ± 187, after: 169 ± 160 µg/dL; TNF-α: before: 22.6 ± 8.95, after: 21.4 ± 9.34 pg/mL; urinary excre-
Fig. 3. Comparison of HOMA-IR, urinary albumin excretion, and estimated glomerular filtration rate with/without ezetimibe treatment.

Values for homeostasis model assessment insulin resistance (HOMA-IR) (left), urinary albumin excretion (middle), and the estimated glomerular filtration rate (right) are shown. Values are the mean ± SD. n = 38 in each group. **p < 0.01

Fig. 4. Comparison of degree of inflammation and oxidative stress, and nitric oxide bioavailability markers with/without ezetimibe treatment.

Values for high-sensitivity C-reactive protein (hs-CRP) (upper left), tumor necrosis factor (TNF)-α (upper right), urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (lower left), and urinary excretion of nitrate and nitrite (NOx) (lower right) are shown. Values are the mean ± SD. n = 38 in each group. *p < 0.05, **p < 0.01

The urinary excretion of 8-OHdG before: 6.11 ± 4.11, after: 5.88 ± 4.04 μg/g creatinine; urinary excretion of NOx before: 22.6 ± 15.5, after: 23.7 ± 18.8 nmol/g creatinine (Fig. 4).

While the effects of ezetimibe were not correlated with a decrease in the LDL-chol level, the decrease in
The serum TNF-α level showed an association ($r = 0.39; p < 0.05$) (Fig. 5).

**Discussion**

The present study showed that ezetimibe decreased not only the LDL-cholesterol levels but also the body weight, waist circumference, blood pressure, and urinary albumin excretion; it also improved insulin resistance. In addition, it decreased the levels of hs-CRP, TNF-α, and urinary 8-OHdG excretion and improved NO bioavailability.

The serum cholesterol level is regulated by synthesis in the liver and absorption from the intestine. Serum LDL-cholesterol levels are often insufficiently controlled by the suppression of cholesterol synthesis with a statin in patients with a genetic disorder or enhanced cholesterol absorption\(^{10,11}\). Ezetimibe is a novel agent that potently inhibits the intestinal absorption of cholesterol from dietary and biliary sources by blocking the Niemann-Pick C1-like 1 protein and is effective for therapy-resistant dyslipidemia\(^{12-14}\). Our data showed that ezetimibe therapy induced a 24.0% reduction in the serum LDL-cholesterol level of our 38 patients; the decrease was 21.2% in patients subjected to ezetimibe monotherapy and 37.8% in those treated with ezetimibe added to on-going statin therapy. In our series, 71.1% of patients treated with ezetimibe alone achieved the treatment goal; the success rate was 86.8% for patients administered ezetimibe added to on-going statin therapy. Others reported a 18.1% and 25.8% decrease in the serum LDL-cholesterol level of patients treated with ezetimibe alone (10 mg/day) and with ezetimibe added to on-going statin therapy, respectively\(^{10,12}\). These data suggest that ezetimibe monotherapy is comparable to statin therapy and that the addition of ezetimibe to on-going statin therapy further reduces the serum LDL-cholesterol level.

We also report that in addition to its lipid-lowering effect, ezetimibe improved metabolic disorders, including obesity, hypertension, insulin resistance, and microalbuminuria. These findings suggest that it produces a decrease in cardiovascular events by ameliorating metabolic syndrome and chronic kidney disease (CKD), crucial risk factors for cardiovascular diseases\(^{15,16}\).

We treated patients at high risk for cardiovascular disease whose renin-angiotensin systems seemed to be activated, as described in patient characteristics. It has been reported that oxidative stress is involved in hypertension or renal dysfunction in conditions with an activated renin-angiotensin system and that NO exerts cardiorenal protective effects\(^{17-19}\). It has also been reported that an NADPH oxidase inhibitor improved diabetes, hyperlipidemia, and hepatic steatosis by reducing oxidative stress in accumulated fat, indicating that reactive oxidative species play a critical role in the pathogenesis of metabolic syndrome\(^{20}\). Therefore, the potential mechanisms of improved metabolic disorders, blood pressure lowering or renal
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protection, including reduced albuminuria, were associated with decreased oxidative stress and increased NO bioavailability. In addition, imbalance of oxidative stress and NO action was also reported to be involved in enhanced inflammation, which is a major pathogenic factor for cardiovascular diseases, including CKD. In our study, ezetimibe might have decreased serum levels of hs-CRP and TNF-α as inflammatory markers through increased NO bioavailability and decreased oxidative stress, which were evaluated by urinary excretion of NOx and 8-OHdG, respectively.

Interestingly, while the changes elicited by ezetimibe in urinary albumin excretion, HOMA-IR, the serum hs-CRP level, and the urinary excretion of 8-OHdG and NOx were not correlated with a decrease in the LDL-cholesterol level, the decrease in the serum TNF-α level was associated. These findings suggest that ezetimibe has cardioprotective actions, including anti-inflammatory and anti-oxidant action and ameliorates NO bioavailability independent of its LDL-cholesterol-lowering effects.

Niemann-Pick C1-like 1 is expressed at a high level not only in the intestine but also in the liver, and Niemann-Pick C1-like 1 receptor is expressed at a very low level in the cardiovascular and renal systems, implying that, unlike statins, ezetimibe does not have direct effects on the cardiovascular and renal systems. It has been shown that nonalcoholic fatty liver disease, including hepatic steatosis, is closely related via enhanced inflammation to insulin resistance, metabolic syndrome, and cardiovascular disease. Tabuchi et al. reported that ezetimibe inhibits the absorption of oxidized cholesterol and its deposition in the liver. Using a rat model of metabolic syndrome, Deushi et al. documented that ezetimibe improved liver steatosis and insulin resistance by decreasing hepatic lipid deposition and fibrosis of the liver and effectively reduced systemic dyslipidemia. These findings suggest that ezetimibe protects against insulin resistance and inflammation by inhibiting the inflow of cholesterol, including oxidized cholesterol into the liver from the intestine and/or by direct effects on the liver.

In conclusion, ezetimibe ameliorates obesity, hypertension, insulin resistance, and microalbuminuria by reducing the degree of inflammation and oxidative stress, and it improves the bioavailability of NO in a LDL-cholesterol reduction-dependent and -independent manner. These results suggest that it may be effective in the treatment of hypercholesteremic patients with metabolic syndrome and CKD. Clinical and basic studies are underway in our laboratory to clarify the detailed mechanisms underlying the beneficial effects of ezetimibe on cardiovascular-renal diseases.

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