ORIGINAL ARTICLE

Colestimide, an anion exchange resin agent, can decrease the number of LDL particles without affecting their size in patients with hyperlipidemia

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KEYWORDS
Colestimide; Hyperlipidemia; Nuclear magnetic resonance; Small dense low-density lipoprotein

Summary
Objectives: Recent studies have demonstrated that not only plasma low-density lipoprotein (LDL) levels, but also the number of small dense LDL particles are involved in the development of arteriosclerosis. Anion exchange resins can reduce plasma LDL levels and affect LDL particle size via increasing triglycerides. In the present study, the effects of short-term colestimide administration on LDL particle size were investigated.

Methods: Obese patients with primary hyperlipidemia (n = 21) were administered 3000 mg/day of colestimide for 1 month and fasting blood was obtained before and after the treatment. LDL particle size and number were measured by nuclear magnetic resonance (NMR) lipoprotein using magnetic resonance spectroscopy.

Results: Levels of plasma LDL cholesterol decreased from 155.5 mg/dl to 128.1 mg/dl (p < 0.001) and levels of apolipoprotein B decreased from 139.2 mg/dl to 120.6 mg/dl (p < 0.001) by colestimide administration. Levels of high-density lipoprotein (HDL) cholesterol and triglyceride were unaltered. LDL particle size did not change, whereas LDL particle numbers decreased from 1920.3 nmol/l to 1568.8 nmol/l (p < 0.01).

Conclusions: Short-term administration of colestimide to patients with hyperlipidemia reduced LDL particle numbers. LDL particle size was not changed.

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Introduction

The method of treating dyslipidemia has been improved dramatically by the introduction of HMG-CoA reductase inhibitors (statins). Anion exchange resin agents, which were once in the mainstream of treatment of dyslipidemia, have been replaced by statins mainly due to the required large dosage and their difficulty for oral use. However, anion exchange resins have a primary preventive effect on coronary artery diseases by lowering low-density lipoprotein (LDL) cholesterol levels [1]. Anion exchange resins can be used in patients with intolerance to statins or in patients where statins have limited efficacy [2]. Anion exchange resins enhance uptake of circulating LDL to cells by increasing the number of LDL receptors in the liver, resulting in reduction of circulating LDL levels [3]. Because liver cholesterol synthesis is enhanced and very (V)LDL can be released to the circulation, the possibility exists that triglyceride, the main component of VLDL, may be increased.

In metabolic syndrome, LDL particle size may become smaller with an increase in triglyceride levels. However, very few studies have been performed on LDL particle size after administration of anion exchange agents. Thus, in this study, the effects of short-term administration of colestimide on LDL particle size were investigated.

Subjects and methods

Patient characteristics

The study protocol was approved by the ethical committee of the Hokkaido University and was in accordance with the Helsinki Declaration. An anion exchange resin formulation (colestimide, 3000 mg/day) was administered for 1 month to 21 obese (as defined by the criteria of Japan Society for the Study of Obesity) untreated patients with primary hyperlipidemia (61.5 ± 9.8 years; male/female = 10/11; body height 159.3 ± 11.0 cm; body weight 71.2 ± 12.0 kg; body mass index 28.0 ± 3.2 kg/m²), who visited the Department of Cardiovascular Medicine, Hokkaido University Hospital, as outpatients and gave informed consent to participate in the study.

Diabetes as defined by the criteria of Japan Diabetes Society was found in 6 patients (28.6%). Patients with a history of ischemic heart disease, peripheral artery obstructive disease, stroke, renal disease, active liver disease, heart failure, and carotid artery stenosis were excluded. Patients with type III hyperlipoproteinemia or patients with triglyceride level >400 mg/dl were also excluded.

Lipid measurements

LDL particle size and serum lipid profile [levels of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, remnant-like particle-cholesterol (RLP-C), and apolipoprotein] were compared before and after administration of colestimide. Concentrations of serum total cholesterol and triglyceride were determined enzymatically. HDL cholesterol was determined by using a homogeneous method (Determiner L HDL-C, Kyowa Medex, Tokyo, Japan), and concentrations of apolipoproteins (apo) A-I, A-II, B, C-II, C-III, and E were determined by turbidometric immunoassays. LDL cholesterol concentration was calculated using the formula of Friedewald et al. [4]. RLP-C was measured by using an immunoadsorbent method (JIMRO II, Otsuka, Tokyo, Japan). LDL particle size was measured by nuclear magnetic resonance (NMR; THE NMR Lipoprofile, LipoMed, Inc., Raleigh, NC, USA) [5]. NMR spectroscopy measures the signal emitted by plasma lipid methyl groups during magnetic resonance scanning. For data evaluation the NMR lipoprotein subtraction were grouped to lipoprotein class. This resulted in the following spectra: VLDL (>27.0 nm); intermediate density lipoprotein (IDL) (23.0—26.9 nm); LDL (18.0—22.9 nm); and HDL (7.3—13.0 nm). LDL includes IDL unless otherwise stated. NMR method is rapid and measures the full spectrum of the lipoprotein subtraction distribution given as lipoprotein particle number for each subclass and the lipoprotein particle size of each lipoprotein subclass at the same time on a small fresh or frozen sample. NMR method does not require non-physiological density gradient centrifugation. Lipoprotein size determined by NMR reflects only lipid fractions and somewhat underestimates as compared to the size determined by polyacrylamide gel electrophoresis method. Instead of expressing subclass levels in lipid mass concentration units (mg/dl cholesterol) as was conventionally done, they are given in particle concentration units (nanomoles of particles per liter, nmol/l) to reflect that NMR is actually measuring LDL particle numbers. Mean particle sizes (nm diameter) were computed as the sum of the diameters of the individual subpopulations multiplied by their relative mass percentages as estimated from the amplitudes of their methyl NMR signals.

Statistical analysis

The biochemical data of the subjects are presented as the mean ± SD. The variables’ differences before to after administration were examined by the Student’s paired t-test. Two-sided p-values < 0.05 were regarded as significant. All analyses were done with SPSS 10.1 (SPSS Inc., Chicago, IL, USA).

Results

The levels of total cholesterol, LDL cholesterol, and apolipoprotein B were significantly improved by the administration of colestimide (Table 1). Level of RLP-C also tended to decrease. Level of triglyceride did not differ significantly before and after treatment. No changes were observed in the levels of HDL cholesterol and apo A1.

For each lipoprotein particle, neither the mean particle size of VLDL nor HDL changed. LDL particles did not decrease in size (Table 2). On the other hand, there was a significant decrease in LDL particle numbers as measured by NMR (Table 2).

Furthermore, as to component percentages of LDL particle size, percentage ratio of small size LDL tended to decrease from 5.0% to 2.5% (Fig. 1) [6]. Percentage ratio of medium size LDL tended to increase from 38.6% to 39.3% and percentage ratio of large size LDL also tended to increase.
Table 1  Changes in plasma cholesterol, lipoproteins, and other lipids before and after colestimide administration in 21 patients.

<table>
<thead>
<tr>
<th></th>
<th>Before (mg/dl) ± SD</th>
<th>After (mg/dl) ± SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>240.3 ± 28.6</td>
<td>208.2 ± 33.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>164.3 ± 57.1</td>
<td>167.5 ± 51.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>38.6 ± 9.6</td>
<td>39.3 ± 9.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>155.5 ± 23.2</td>
<td>128.1 ± 28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>126.0 ± 20.3</td>
<td>128.8 ± 20.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apolipoprotein AII</td>
<td>26.5 ± 5.1</td>
<td>25.3 ± 5.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>139.2 ± 17.1</td>
<td>120.6 ± 18.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein CII</td>
<td>5.6 ± 2.1</td>
<td>5.5 ± 1.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apolipoprotein CIII</td>
<td>10.5 ± 4.0</td>
<td>11.3 ± 3.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>4.9 ± 0.9</td>
<td>4.6 ± 1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>RLP-C</td>
<td>8.1 ± 3.2</td>
<td>7.1 ± 2.1</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein; RLP, remnant-like particle.

Table 2  Changes in mean size and numbers of lipoprotein particles before and after colestimide administration in patients.

<table>
<thead>
<tr>
<th></th>
<th>Before ± SD</th>
<th>After ± SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL particle size (nm)</td>
<td>42.0 ± 6.6</td>
<td>43.6 ± 6.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL particle size (nm)</td>
<td>21.2 ± 0.7</td>
<td>21.2 ± 0.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL particle size (nm)</td>
<td>9.0 ± 0.6</td>
<td>9.1 ± 0.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL particle numbers (nmol/l)</td>
<td>1920.3 ± 454.8</td>
<td>1568.8 ± 286.2</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Figure 1  Changes in lipoprotein content percentage ratios in each low-density lipoprotein (LDL) particle size category before and after the administration of colestimide. Percentage ratio of small size LDL tended to decrease (5.0—2.5%). Percentage ratio of medium LDL (38.6—39.3%) and large size LDL (50.3—52.1%) tended to increase after treatment. Percentage ratio of intermediate density lipoprotein (IDL) did not show any changes (6.1—6.1%). For data evaluation, the nuclear magnetic resonance lipoprotein subfractions were grouped into size groups for LDL lipoprotein class. This resulted in the following spectra: IDL (23.0—26.9 nm); large size LDL (21.3—22.9 nm); medium LDL (19.8—21.2 nm); and small LDL (L1, 18.0—19.7 nm) [6].

from 50.3% to 52.1% after treatment. Percentage ratio of IDL did not change from 6.1% to 6.1%.

Discussion

Colestimide is non-absorbable and enhances excretion of bile acids in feces via absorption in the intestinal tract. This causes inhibition of intestinal circulation of bile acids, depletion of the cholesterol pool in the liver, increase in LDL receptors, and decrease in circulating LDL cholesterol level. Further, it is believed that inhibition of the absorption of cholesterol derived from food plays a role in the lowering of LDL cholesterol level. On the other hand, the possibility exists that LDL particles might decrease in size with increase in triglyceride due to re-synthesis of VLDL. Small dense LDL, which is small and high in density, is a risk factor for ischemic heart disease [7] and simvastatin and bezafibrate have beneficial effects on LDL particle size [8,9]. However, for colestimide, there have been almost no reports on investigations of its effects on lipid profile, especially LDL particle size, such as in large-scale clinical trials. In the present study, LDL particles were directly measured using the NMR method before and after the administration of colestimide, making this the first report on changes in such particles induced by colestimide. Despite a brief administration period, colestimide lowered LDL cholesterol but also increased triglyceride slightly. LDL particle size was not changed. In this study LDL particle number was significantly decreased to 81.7% of baseline value after treatment and LDL cholesterol was reduced to 82.8% of baseline value after treatment. Based on these data it was expected that LDL particle size was not changed. NMR data confirmed that the LDL particle size was indeed not changed.

A significant increase in LDL particle size is reported with colestimide administration in patients with normal fasting blood glucose [10]. LDL particle size has been generally measured using polyacrylamide gradient gel electrophoresis (GGE) [11]. Small dense LDL particle size measured by the GGE method was 20.5 nm or less, which was smaller than that measured using NMR method by about 5 nm [12]. Yoshino et al. reported a significant decrease in mobility (Rf) value...
after colestimide administration, indicating increase in LDL particle size [10]. They used a simple LipoPrint LDL system (Quantimetrix, Redondo Beach, CA, USA), for which a negative correlation to those obtained with the GGE method has been shown. They also assumed that the LDL fraction with Rf of 0.40 or higher contained small dense LDL [13]. In their system Rf of 0.40 or higher corresponds with small dense LDL with a particle size of 25.5 nm or below. They only assumed that significant reduction of Rf values after colestimide administration implicated an increase in LDL particle size. Thus, direct evidence of the improvement in LDL particle size was not present in their study. The decrease rate in small size LDL appeared to be larger than that in RLP-C. Colestimide ameliorates glucose metabolism in mice [14]. A significant increase in LDL particle size is reported with colestimide administration in patients with normal fasting blood glucose [10]. The patients in this study were obese, and diabetes was found in 6 patients. The differences in clinical characteristics may have affected the response of LDL to colestimide. Further investigation with larger number of patients will be necessary with respect to the effect on LDL and non-lipid parameters in the subgroup with high RLP-C or diabetes.

Limitations

In this study, mean LDL particle size before colestimide administration was not particularly small (21.2 nm), and the content of large LDL in LDL was 50% or higher while the content of small LDL in LDL was quite small at 5.0%. In addition, it is speculated that the short period of administration and relatively small number of subjects may have contributed to the lack of demonstration of increase in particle size. It has been suggested that the increase in triglyceride level by colestimide administration does not decrease LDL particle size. Elucidation of the effects of simvastatin and bezafibrate, which have beneficial effects on LDL particle size [8,9] and on LDL particle numbers will be also necessary.

Conclusion

In summary, our findings suggest that colestimide not only lowers the levels of LDL cholesterol and apolipoprotein B, but also reduces LDL particle numbers without reducing LDL particle size. Statins may cause allergy or may not be sufficiently effective in some patients. Colestimide may be effective in improving LDL cholesterol in such patients. LDL particle size and number provide independent measures of atherogenicity and are strong predictors of cardiovascular disease [15]. Because colestimide exhibited insufficient efficacy for increase in LDL particle size, further investigation is required.

Acknowledgements

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