Effect of ezetimibe on lipoprotein subfraction concentrations: the role of atorvastatin pretreatment

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Abstract

Introduction: Recently published data indicate that the effect of ezetimibe on lipoprotein subfraction distribution in patients receiving statin therapy may differ substantially from that observed in patients treated with ezetimibe monotherapy. The aim of our study was to directly compare the effects of ezetimibe added to established atorvastatin treatment on lipoprotein subfractions with those obtained by ezetimibe monotherapy.

Material and methods: Forty dyslipidaemic patients who failed to reach their assigned LDL-C target while on atorvastatin therapy (20 mg/day) for at least 6 months were included in the study. Ezetimibe (10 mg/day) was added to atorvastatin in all patients. The concentrations of the individual lipoprotein subfractions were determined using the Lipoprint method at baseline (prior to the addition of ezetimibe) as well as after 16 weeks of combination treatment. The changes in lipoprotein subfraction concentrations were compared with those observed in 40 age- and sex-matched statin-naïve patients receiving ezetimibe monotherapy for 16 weeks.

Results: Ezetimibe administration reduced VLDL concentrations either when used as monotherapy or when added to established atorvastatin treatment. However, in contrast to ezetimibe monotherapy, which reduced the concentrations of all LDL subfractions, the addition of ezetimibe in patients receiving atorvastatin decreased LDL cholesterol values exclusively by reducing the concentrations of large, buoyant LDL subfractions. In addition, while ezetimibe monotherapy reduced mainly the concentrations of small, dense LDL subspecies, the addition of ezetimibe in patients receiving atorvastatin equally reduced the concentration of all HDL particles.

Conclusions: The effect of ezetimibe on lipoprotein subfractions is significantly affected by previous atorvastatin treatment.

Key words: small, dense LDL, LDL subfractions, HDL subfractions, triglycerides.

Introduction

It is well known that low-density lipoproteins (LDL) do not represent a collection of identical particles but rather are composed of discrete subfractions that differ with respect to their size, density, composition, charge and other physicochemical properties [1]. These differences are, at least in part, responsible for the differences observed in the biological behaviour of LDL subfractions and more specifically in their ability to promote...
atherogenesis. Experimental studies have shown that small, dense LDL particles are more atherogenic than large, buoyant LDL subfractions [2-5] and these observations were subsequently confirmed by the results of large clinical trials. Indeed, the Quebec cardiovascular study as well as the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) results revealed a linear relationship between the concentrations of small, dense LDL particles and the risk of subsequent development of cardiovascular events [6, 7].

Epidemiological studies have shown that high-density lipoprotein (HDL) cholesterol levels are inversely related to the risk for coronary heart disease [8]. However, the subsequent subfractionation of HDLs revealed that the individual HDL subfractions are not equally atheroprotective. Indeed, the antiatherogenic properties of HDLs are mainly attributed to their dense subfractions, which are more efficient cholesterol acceptors and exhibit increased anti-oxidative activity [9].

Ezetimibe is the first member of a new class of selective cholesterol absorption inhibitors. Recently published data indicate that ezetimibe inhibits the transport of cholesterol across the brush border of the intestinal wall by inhibiting the function of Niemann-Pick C 1-like protein [10]. This inhibition decreases the cholesterol content of hepatocytes and results in an upregulation of LDL receptors which, in turn, reduces the serum concentrations of LDL cholesterol. The efficacy of ezetimibe as an LDL-lowering agent (when used either as monotherapy or in combination with other hypolipidaemic compounds, such as statins or fibrates) has been extensively studied [11-13]. However, so far there are only limited data on the effect of ezetimibe on the LDL subfraction profile. More specifically we and others have shown that ezetimibe monotherapy exerts its LDL-lowering effects by reducing the concentrations of all individual LDL subfractions [14, 15]. In addition, in a previous study we showed that ezetimibe monotherapy may reduce the serum levels of HDL mainly by reducing the concentration of dense HDL subfractions [14]. Nevertheless, recent studies indicate that the effect of ezetimibe on lipoprotein subfraction profile may substantially differ from this pattern when the drug is used in individuals treated with other lipid-lowering modalities such as LDL apheresis and statins [16]. Therefore, we undertook the present study to directly compare the effects of ezetimibe added to established atorvastatin treatment on lipoprotein subfractions with those obtained by ezetimibe monotherapy.

Material and methods

Patients

Forty unrelated, consecutive patients who failed to reach their assigned LDL-C target while on atorvastatin therapy (20 mg/day) for at least 6 months were included in the study (combination group). Individuals receiving medications other than atorvastatin that may affect lipid metabolism were excluded. LDL-C target calculations were based on National Cholesterol Education Program (NCEP) guidelines [17]. None of the study participants had a history or clinical and electrocardiographic evidence of coronary heart disease or equivalents (symptomatic carotid artery disease, aortic aneurysm, peripheral arterial disease or diabetes mellitus). All study participants were given 10 mg of ezetimibe plus atorvastatin (20 mg) in a single morning dose. Serum lipids and apolipoproteins as well as lipoprotein subfraction concentrations were measured at baseline (after 6 months of atorvastatin therapy but before the initiation of ezetimibe) as well as after 16 weeks of combination treatment. The effects of the addition of ezetimibe on established atorvastatin therapy on lipoprotein subfractions were compared with those obtained by ezetimibe monotherapy. For this reason we compared our study participants with a group of 40 age- and sex-matched individuals with primary dyslipidaemias who received 10 mg of ezetimibe for 16 weeks (monotherapy group). These individuals were a subgroup of the study population of a previous study that tested the effect of ezetimibe monotherapy on lipoprotein subfraction concentrations [14]. Compliance with the medication was assessed by questionnaire and tablet counts. None of the study participants missed more than 3 doses during the active treatment period. The consumption of phytosterol-enriched products was not allowed during the study period. All study individuals gave their written informed consent prior to enrolment and the study was approved by the Scientific Committee of the University Hospital of Ioannina.

Analytical methods

All lipid and lipoprotein determinations were carried out after an overnight fast. Serum levels of total cholesterol, HDL-C and triglycerides were determined enzymatically using an Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). Serum LDL-C was calculated using the Friedewald formula. Serum apolipoproteins AI and B levels were measured with a Behring Holding GmbH (Liederbach, Germany).

Lipoprotein subfraction analysis

LDL subfraction analysis

Electrophoresis was performed using high resolution 3% polyacrylamide tube gel and Lipoprint LDL System (Quantimetrix, Redondo Beach, CA) as previously described [14].

HDL subfraction analysis

The cholesterol content of HDL subfractions was determined by Lipoprint HDL System (Quantimetrix, Redondo Beach, CA) as previously described [14].
Statistical analysis

Data are presented as mean ± SD unless otherwise stated. All variables were tested for normality with the Kolmogorov-Smirnov tests. No significant deviations from normality were found with the exception of triglycerides, which were log-transformed before analysis. A paired t-test was used for comparisons between baseline and post-treatment values, while analysis of covariance (ANCOVA) taking into account the baseline values of each parameter as a covariate was used for comparisons between the two treatment groups. In cases of multiple comparisons the Bonferroni correction was applied. Linear regression analysis was used for the assessment of the correlations between the changes in the concentrations in sdLDL and those in serum lipid values. Multiple regression analysis was used for the determination of the factors that may affect the serum concentrations of sdLDL at baseline.

Results

No differences in age (54.6±16.1 vs. 51.9±16.1 years for monotherapy and combination therapy group, respectively), sex distribution, body mass index (26.9±3.9 vs. 26.4±3.7 kg/m² for monotherapy and combination therapy group, respectively) were found between the two study groups. However, patients who received ezetimibe monotherapy (monotherapy group) exhibited significantly higher levels of total, LDL and HDL cholesterol at baseline compared to individuals who received ezetimibe on top of previous atorvastatin treatment (combination group). No differences were observed in the baseline serum levels of triglycerides, apolipoprotein AI and apolipoprotein B between the two study groups (Table I).

As shown in Table I, ezetimibe induced a decrease in the serum levels of total and LDL cholesterol either when used as monotherapy or when added to previous atorvastatin treatment. However, the reductions in the concentrations of these parameters were significantly greater in the combination group than those observed in the ezetimibe monotherapy group. Ezetimibe administration also significantly reduced the serum levels of triglycerides as well as the concentrations of HDL cholesterol and apolipoprotein B. No differences in the magnitude of these reductions were observed between the two study groups. Finally, ezetimibe did not significantly affect the serum levels of apolipoprotein AI in both study groups.

Table II displays the concentrations of lipoprotein subfractions in both study groups at baseline. Patients assigned to receive ezetimibe monotherapy had significantly higher levels of cholesterol in large LDL subfractions. On the other hand, no significant differences were observed in the concentrations of VLDL, small, dense LDL particles or HDL subspecies between the study groups. As shown in Table II, ezetimibe administration induced a significant decrease in VLDL in both study groups; however, this decrease was more pronounced in the combination group. The subfractionation of LDLs revealed a decrease in the concentration of all LDL subfractions after ezetimibe monotherapy; this was more pronounced in the small, dense LDL subfractions. On the other hand, in the combination group the reduction in LDL cholesterol levels was due to a significant decrease in the concentrations of large LDL subfractions, whereas the concentrations of sdLDL subfractions were only marginally decreased. Finally, in the ezetimibe monotherapy group a significant reduction in the concentrations of dense HDL subfractions also occurred, whereas in the combination therapy group the serum levels of all HDL subfractions were equally reduced. However, the percentage changes in the concentrations of HDL subfractions did not differ significantly between the two study groups.

| Table I. Effect of ezetimibe on lipid and apolipoprotein values |
|-------------------|-------------------|-------------------|
| **Monotherapy group** | **Combination therapy group** |
|                      | Baseline | Post-treatment | % change | Baseline | Post-treatment | % change |
| Total cholesterol (mg/dl) | 267±40 | 225±36 | −15.1 (−11.7 to −18.5) | 240±44* | 184±40 | −22.7 (−19.5 to −25.8)* |
| Triglycerides (mg/dl) | 146±72 | 129±52 | −4.4 (−3.1 to +4.3) | 129±62 | 109±53 | −10.3 (−2.3 to −18.4) |
| HDL cholesterol (mg/dl) | 62±13 | 58±12 | −5.8 (−2.2 to −9.4) | 56±11* | 51±12 | −9.6 (−5.3 to −12.9) |
| LDL cholesterol (mg/dl) | 176±28 | 141±27 | −18.6 (−13.7 to −23.5) | 158±36* | 111±34 | −28.5 (−23.9 to −33.1)* |
| Apolipoprotein AI (mg/dl) | 157±28 | 152±24 | −0.8 (−5.9 to +4.3) | 144±21 | 139±25 | −0.7 (−5.8 to +4.2) |
| Apolipoprotein B (mg/dl) | 113±23 | 94±22 | −11.1 (−2.5 to −19.6) | 111±30 | 84±25 | −23.2 (−14.8 to −31.5) |

Lipid and apolipoprotein values are presented as mean ± standard deviation. The percentage changes are given as mean (95% CI). Analysis of covariance (ANCOVA) taking into account the baseline values of each parameter as a covariate was used for comparisons between the percentage changes observed in the two study groups, while a t-test was used to compare the baseline lipid and apolipoprotein values. In either case the Bonferroni correction was applied.

* − significantly different compared to monotherapy group.
Effect of ezetimibe on lipoprotein subfractions

**Table II.** Effect of ezetimibe on the cholesterol content of lipoprotein subfractions (in mg/dl)

<table>
<thead>
<tr>
<th>Lipoprotein Subfraction</th>
<th>Monotherapy group</th>
<th>Combination therapy group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (mg/dl)</td>
<td>Post-treatment (mg/dl)</td>
</tr>
<tr>
<td>VLDL</td>
<td>53.9±12.3</td>
<td>44.4±12.7</td>
</tr>
<tr>
<td>Large LDL</td>
<td>136.4±22.6</td>
<td>112.4±22.4</td>
</tr>
<tr>
<td>Small, dense LDL</td>
<td>7.6±4.9</td>
<td>4.5±4.9</td>
</tr>
<tr>
<td>Large HDL</td>
<td>22.2±10.2</td>
<td>22.1±11.8</td>
</tr>
<tr>
<td>Intermediate density HDL</td>
<td>31.9±8.2</td>
<td>31.5±5.9</td>
</tr>
<tr>
<td>Small, dense HDL</td>
<td>10.2±2.9</td>
<td>8.2±2.8</td>
</tr>
</tbody>
</table>

Lipoprotein subfraction values are presented as mean ± standard deviation. The percentage changes are given as mean (95% CI). Analysis of covariance (ANCOVA) taking into account the baseline values of each parameter as a covariate was used for comparisons between the percentage changes observed in the two study groups, while a t-test was used to compare the baseline lipid and apolipoprotein values. In either case the Bonferroni correction was applied.

* – significantly different compared to monotherapy group

Multiple regression analysis (that included triglycerides, LDL-C, HDL-C and serum concentrations of apolipoproteins A1 and B as predictors) revealed that the serum level of triglycerides was the only significant determinant of sdLDL concentrations in both study groups (beta values 0.81 and 0.53 for the monotherapy and combination therapy group, respectively; p<0.001 for both correlations). On the other hand the LDL-C values were the only important determinant of the concentrations of large LDL subspecies (beta values 0.80 and 0.63 for the monotherapy and combination therapy group, respectively; p<0.001 for both correlations).

As expected, the reduction in triglyceride levels was the most important predictor of the reductions in sdLDL concentrations in the entire study population. However, this relationship was significantly influenced by the concentration of triglycerides at baseline. Thus, in individuals with baseline triglyceride values greater than 120 mg/dl a strong correlation between the reductions in triglycerides and those in sdLDL concentrations was observed (Figure 1A). On the other hand, in study subjects with baseline triglycerides below this threshold, the reductions in triglyceride concentrations were not accompanied by important decreases in sdLDL values (Figure 1B). The same phenomenon was observed when the two study groups were tested separately. However, for any given reduction in triglycerides the individuals with baseline triglyceride values above 120 mg/dl who received ezetimibe monotherapy exhibited greater reductions in the concentrations of sdLDL compared to individuals with similar triglyceride values who received combination therapy (Figure 2). Finally, the changes in LDL-C values were the most important determinant of the corresponding changes in the concentrations of large LDL subfractions in both study groups, whereas the reductions in HDL subspecies were not significantly correlated with those in serum lipid levels.

**Figure 1.** Correlation between the changes in triglyceride values and those in small, dense LDL subfraction concentrations in patients with baseline triglyceride values greater (A) or lower (B) than 150 mg/dl. Negative values represent increases in the concentrations of triglycerides or small, dense LDL particles.
Discussion

Previously published studies have shown that the evaluation of serum lipoprotein subfraction profile may substantially contribute to the determination of the total cardiovascular risk [6, 7, 18]. Ezetimibe, an inhibitor of the intestinal absorption of cholesterol, is increasingly used (alone or in combination with statins) in the treatment of hypercholesterolaemia. However, although the hypolipidaemic efficacy of ezetimibe has been extensively studied, its effect on the concentration and relative distribution of lipoprotein subfractions remains ill defined. In the present study we directly compared the effects of ezetimibe monotherapy on lipoprotein subfractions with those obtained by the addition of ezetimibe to established atorvastatin therapy. In agreement with previously published data we found that ezetimibe monotherapy reduces LDL cholesterol by decreasing the concentrations of all LDL subfractions, whereas the ezetimibe-induced decrease in HDL cholesterol levels is mainly attributed to the reduction in dense HDL subfraction concentrations. In contrast, the reduction in LDL cholesterol levels induced by the addition of ezetimibe to atorvastatin is exclusively due to a decrease in the concentrations of large LDL subfractions, whereas the concentrations of sdLDL are not significantly affected. Additionally, in contrast to ezetimibe monotherapy, ezetimibe added on a background of atorvastatin equally reduces the concentration of all HDL subspecies.

Kinetic studies have shown that ezetimibe monotherapy as well as ezetimibe-statin combinations reduces VLDL and LDL concentrations mainly by decreasing the production of VLDL and by increasing the LDL receptor-mediated catabolism of LDL particles, respectively [19, 20]. However, although the reduction in the concentrations of large LDL subfractions observed in both groups of our patients may represent a consequence of the increased fractional catabolic rate of LDL particles, this mechanism cannot sufficiently explain the drug-induced changes in the concentrations of sdLDL. Indeed, sdLDL subfractions exhibit decreased affinity for LDL receptors and their serum concentrations are regulated by poorly understood mechanisms [5].

Previously published studies have shown that the serum levels of triglycerides represent the most important single determinant of sdLDL subfraction concentration [21, 22]. Thus, patients with high serum triglyceride values have been shown to exhibit higher concentrations of small dense LDL particles as compared to patients with lower triglyceride values. In this context, a critical concentration of 120 mg/dl has been proposed from some investigators to exist for the classification of patients as having “high” or “low” triglyceride values [21]. In agreement with this notion we found a significant correlation between triglyceride and sdLDL concentrations in both study groups. In addition, our findings are consistent with the existence of a threshold in triglyceride concentration above which the sdLDL formation procedure is accelerated. Indeed, in patients with baseline triglyceride values greater than 120 mg/dl the reduction in sdLDL concentration parallels that in triglycerides, whereas the reduction of triglyceride levels below this threshold is not followed by significant changes in the concentrations of LDL subspecies. As a consequence, the limited reduction in the concentration of sdLDL in patients receiving ezetimibe on a background of atorvastatin could not be ascribed to previous atorvastatin treatment but rather, at least in part, to the lower baseline triglyceride concentrations in this patient group. However, although this mechanism is pathophysiologically plausible, in cannot explain the greater magnitude of the reduction in sdLDL in hypertriglyceridaemic patients receiving ezetimibe monotherapy compared to individuals with the same triglyceride values who received combination therapy.

The regulation of LDL particles’ distribution is a very complicated process that involves the production and catabolism of triglyceride-rich lipoproteins as well as the intravascular remodelling and catabolism of the LDL particles [23]. A number of enzymes [lipoprotein lipase, hepatic lipase, lecithin-cholesterol acyltransferase (LCAT)] and transfer proteins [cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP)] have been implicated in this procedure, the details of which still remain indeterminate [23]. Previous studies have shown that atorvastatin treatment may significantly affect the activities of most enzymes involved in the formation of sdLDL [24–26]. Thus, it can be speculated that the partial inhibition of CETP and/or hepatic lipase activity (due to atorvastatin pretreatment) may limit the sdLDL-lowering effect of triglyceride reduction. In other words in individuals with impaired ability to synthesize sdLDL particles
the administration of ezetimibe may fail to further suppress the formation of these particles despite the significant reduction in triglyceride values. Consistent with this hypothesis is the finding that torcetrapib (a selective CETP inhibitor), which sufficiently reduces the concentration of sdLDL when used as monotherapy, failed to decrease the concentration of these particles when added to previous atorvastatin treatment [27].

Another interesting finding of our study is that ezetimibe, alone or on a background of atorvastatin, significantly reduces the concentration of HDL cholesterol. However, by contrast with ezetimibe monotherapy, which reduces only the dense subfractions of HDL, ezetimibe added to established atorvastatin treatment equally affects the serum levels of all HDL subfractions. Since the reduction in HDL cholesterol levels after ezetimibe administration was not consistently observed in previous studies it may be due to the limited number of patients enrolled in our study. However, we believe that further studies are needed to delineate the effects of ezetimibe on HDL metabolism and to characterize the pathophysiological mechanisms that may underlie these effects.

In conclusions, the effect of ezetimibe on lipoprotein subfractions is significantly affected by previous atorvastatin treatment. In contrast to ezetimibe monotherapy, which reduces all LDL subfractions, ezetimibe administered to individuals already receiving conventional doses of atorvastatin decreases exclusively the concentrations of large LDL subfractions without affecting the serum levels of sdLDL. Although these differences might be explained on the basis of different baseline triglyceride values, the atorvastatin-induced partial inhibition of the enzymes involved in the formation of sdLDL may also play a contributory role. Additionally, while ezetimibe monotherapy decreases HDLs mainly by reducing the concentrations of dense HDL particles, ezetimibe on a background of atorvastatin equally reduces the concentrations of all HDL subfractions. It is evident that further studies are required to completely characterize the effect of ezetimibe on HDL metabolism.

References


