Chitotriosidase Genotype and Serum Activity in Subjects With Combined Hyperlipidemia: Effect of the Lipid-Lowering Agents, Atorvastatin and Bezafibrate


Chitotriosidase, an enzyme involved in the degradation of chitin-containing pathogens with unclear function in humans, has been proposed as a marker of lipid accumulation in macrophages in different lipid-storage diseases, including atherosclerosis. To evaluate (1) if lipid-lowering treatment could modify serum chitotriosidase activity and (2) be useful in monitoring lipid-lowering treatment, we have analyzed this enzyme activity in the participants in the Atozvastatin Versus Bezafibrate in Mixed Hyperlipidemia (ATOMIX) study, a double-blind, comparative, and randomized study comparing the efficacy of atorvastatin and bezafibrate in mixed hyperlipidemia. Because a common genetic deficiency of chitotriosidase modifies serum chitotriosidase activity, this genetic variation was also studied. Seven subjects of 116 (6.03%) were homozygous, and 46 (39.6%) were heterozygous for the defective allele. Mean serum chitotriosidase activity correlated with allele dosage, as it was found to be of 0, 59.8 $\pm$ 52.6 and 81.2 $\pm$ 41.6 nmol/mL/h, in homozygotes for the defective allele, heterozygotes, and homozygotes for the wild-type allele, respectively ($P = .0011$ for the difference between the last 2 groups). However, this enzyme activity was not found to correlate with lipid levels before and after treatment with either atorvastatin or bezafibrate, and neither with the intensity of the lipid lowering. These results do not support the use of serum chitotriosidase activity as a biologic marker of atherosclerotic plaque modification related to hypolipidemic treatment.

ACTIVATED MACROPHAGES inside the atherosclerotic lesion play a very important role in the evolution of the vascular plaque. The lipid accumulation in macrophages contribute to their morphologic transformation into foam cells. This lipid accumulation also promotes the expression of different genes in the macrophage that can interfere in the atherosclerotic process. One of these gene products, the apolipoprotein (apo) E, has been extensively studied, and these studies have provided in vivo demonstration that macrophage-derived apo E carry out a protective function in atherosclerosis.

Chitotriosidase belongs to the chitinase family, a group of enzymes with the capacity to hydrolyze chitin. Chitinases are extensively distributed in a wide variety of animal species and, due to their established antifungal action in plants, they are probably involved in the degradation of chitin-containing pathogens. Chitotriosidase in humans is synthesized exclusively by activated macrophages, and its activity has been proposed as a biochemical marker of macrophage accumulation in several lysosomal diseases, especially in Gaucher disease, in which chitotriosidase activity is more than 660-fold that of controls. Interestingly, plasma chitotriosidase levels decrease more than 50% very rapidly, within 10 to 20 weeks, after the initiation of enzyme replacement therapy with alglucerase in Gaucher disease patients, and this reduction precedes the clinical response to the treatment.

Our group has recently shown a very high correlation among plasma lipids, macrophage-derived apo E, and chitotriosidase activity in serum of patients with Gaucher disease. Moreover, Boot et al have recently shown that chitotriosidase activity was elevated up to 55-fold in extracts of atherosclerotic tissue, showing a clear connection between chitotriosidase expression and lipid-laden macrophages inside the atherosclerotic vessel wall. All together, these data suggest that serum chitotriosidase could be used also as a marker of activated macrophages in vivo.

Because an important part of the protein production of activated macrophages in vitro is derived from chitotriosidase, this chitotriosidase production is well related to their lipid accumulation both in Gaucher disease and in atherosclerotic lesions, this production is rapidly reduced with treatment, and lipid-lowering drugs can reduce macrophage number and macrophage lipid content inside the plaques. We speculate that serum chitotriosidase activity could be related to the number of activated lipid-laden macrophages, and that lipid lowering treatment could modify this chitotriosidase activity in serum of hyperlipidemic patients, and in that way, this hypothetical variation could help to monitor lipid-lowering treatment.

To evaluate our hypothesis, we studied the serum chitotriosidase activity and a common chitotriosidase gene polymorphism, known to modify chitotriosidase activity, before and after lipid-lowering treatment in a group of subjects enrolled in the Atozvastatin Versus Bezafibrate in Mixed Hyperlipidemia (ATOMIX) study, a 1-year, double-blind, comparative, and randomized study comparing the efficacy of atorvastatin and bezafibrate in mixed hyperlipidemia. The chitotriosidase gene polymorphism is a 24-bp duplication in exon 10 resulting in a splicing modification and the generation of a mRNA with an in-frame deletion of 87 nucleotides. This genetic variance is responsible for the recessive inherited deficiency in chitotriosidase activity that is frequently found in different populations.
MATERIALS AND METHODS

Subjects

A complete description of the ATOMIX study has been described elsewhere. Briefly, 138 patients, men and women, aged 18 to 80 years, were selected because primary mixed hyperlipidemia, defined by a mean (of 2 consecutive analyses under hypolipidemic diet) level of triglycerides less than 500 and ≥200 mg/dL, in addition to low-density lipoprotein (LDL)-cholesterol less than 250 and greater than 190, 180, 160, or 135 mg/dL, depending on global risk status according to European Atherosclerosis Society (EAS) recommendations. Qualifying patients were randomly assigned either to 10 to 40 mg atorvastatin (according to EAS LDL-cholesterol target guidelines) or 400 mg bezafibrate (slow release) once daily, and they were followed for 1 year. The investigational review board of each institution approved the study. All patients gave written informed consent.

DNA Analysis

To study the common genetic deficiency in chitotriosidase activity, genomic DNA was isolated from peripheral blood cells from samples obtained at the moment of enrollment using the method of Miller et al. DNA was quantified and diluted to a final concentration of 100 ng/µL to be used in polymerase chain reaction (PCR) analysis. The duplication mutation analysis in the chitotriosidase gene was performed by PCR followed by electrophoresis of the amplified fragments, as described by Boot et al.

Chitotriosidase Activity

Chitotriosidase activity was determined at 37°C in a final volume of 210 µL with 22 mmol/L fluorogenic substrate 4-methylumbelliferyl β-D-N,N′,N′-triacetylchitotriosidase (Sigma, St Louis, MO) in McIlvain buffer (100 mmol/L citric acid, 200 mmol/L sodium phosphate, pH 5.2). The reaction was stopped with 2 mL of 0.3 mol/L glycine-NaOH buffer (pH 10.6). Fluorescent 4-methylumbelliflorone was measured with a fluorimeter (Kontron Instruments, Watford, UK) at 366 nm excitation and 446 nm emission. The procedure were performed as described by Boot et al.

Serum chitotriosidase activity was measured by duplication in 2 serum samples obtained previously to drug treatment and after 6 months of hypolipidemic treatment. The variation of the chitotriosidase activity in those samples made by duplication was less than 5% in all cases.

Statistical Analysis

Chitotriosidase genotype information was obtained from 116 subjects. Seven of them were homozygous for the defective allele, without detectable serum chitotriosidase activity and were excluded for further analysis. A total of 90 subjects, 43 on atorvastatin and 47 on bezafibrate, had information about genotype and serum activity of chitotriosidase.

RESULTS

Chitotriosidase Genotype

The frequencies of the major and minor quitotriosidase alleles were 0.74 and 0.26, respectively (Table 1). Seven subjects were homozygous for the defective allele, and 63 subjects were homozygous for the normal allele. The genotype distribution was in Hardy-Weinberg equilibrium, and there were no allelic differences between subjects on atorvastatin and bezafibrate treatments.

Chitotriosidase Genotype and Serum Chitotriosidase Activity

No detectable serum chitotriosidase activity was found in the 7 subjects homozygous for the defective allele. For this reason, these 7 subjects were excluded in further analysis.

Serum chitotriosidase activity was significantly higher in homozygous subjects for the major allele than in heterozygous subjects for the defective allele: mean 81.2 ± 41.6 nmol/mL/h v 59.8 ± 52.6 nmol/mL/h, P < .01 (Table 1).

Chitotriosidase Activity and Lipid and Lipoprotein Concentration

Mean chitotriosidase activity was 72.6 ± 46.0 nmol/mL/h at baseline, and there was no difference between chitotriosidase activity and treatment group: 76.2 ± 44.6 nmol/mL/h in the atorvastatin group and 69.2 ± 47.5 nmol/mL/h in the bezafibrate group (P = .3283).

Mean chitotriosidase activity globally and per treatment groups, as well as mean concentrations of lipid parameters, are detailed in Table 2.

Chitotriosidase activity remained fairly constant throughout the study. Mean differences in serum chitotriosidase activity were not normally distributed, and for this reason, we used nonparametric analyses for comparisons and the median as summary of results. The Mann-Whitney nonparametric test was performed to compare the effects of chitotriosidase activity between alleles and treatments (atorvastatin or bezafibrate), the Wilcoxon Signed-Rank test to compare the final value versus baseline, and the Spearman correlation coefficient to evaluate the relationship between quantitative variables. Version 6.12 of SAS (SAS Institute, Cary, NC) was used for analysis and summarization.

A modified intent-to-treat (MITT) analysis was performed with data from patients who were randomized to treatment, met the preestablished diagnostic criteria for inclusion, were known to take at least 1 dose of the drugs tested, and provided any follow-up data for LDL cholesterol.

| Table 1. Chitotriosidase Genotype Distribution, Allele Frequency, and Chitotriosidase Activity in the ATOMIX Study |
|-------------------------------------|-------------------|-----------------|------------------|-----------------|
| No. of Subjects (N = 116) Genotype (%) Allele Frequency Chitotriosidase Activitya (Median (95% CI) Mean ± SD) |
| Wild-type 63 54.3 74 (65-86) nmol/mL/h 81.2 ± 41.6 |
| Heterozygous defective allele 46 39.6 42 (36-65) nmol/mL/h 59.8 ± 52.6 |
| Homozygous defective allele 7 6.03 0 nmol/mL/h |
| Wild-type allele 0.74 |
| Defective allele 0.26 |

Abbreviation: CI confidence interval.

*aChitotriosidase activity of the 97 available subjects for enzyme analysis at baseline.
between treatment groups after 6 months. There was no difference in serum chitotriosidase activity group as a whole, and in either treatment group or chitotriosidase genotype group.

Table 2. Chitotriosidase Activity and Lipid and Lipoprotein Plasma Concentrations in the ATOMIX Study at Baseline, After Treatment, and According to the Lipid-Lowering Drug Group

<table>
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<tr>
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<th>Whole Group (n = 90)</th>
<th>Atorvastatin Group (n = 43)</th>
<th>Bezafibrate Group (n = 47)</th>
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<td></td>
<td>Baseline</td>
<td>On Treatment</td>
<td>P</td>
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<tr>
<td>Ct activity, nmol/mL/h</td>
<td>72.6 ± 46.0</td>
<td>72.2 ± 39.9 NS</td>
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<td>Total cholesterol, mg/dL</td>
<td>280 ± 29</td>
<td>234 ± 46 .0001</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>277 ± 68</td>
<td>208 ± 83 .0001</td>
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<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>187 ± 26</td>
<td>153 ± 40 .0001</td>
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<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>39 ± 7</td>
<td>46 ± 11 .0001</td>
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NOTE. Values are means ± SD.
Abbreviations: Ct, chitotriosidase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NS, not significant.

activity between the baseline period and after 6 months of hypolipidemic drug treatment were 1 nmol/mL/h (for non-change P = .2110) in the atorvastatin group and 0 nmol/mL/h (for nonchange P = .7428) in the bezafibrate group. There was no difference in serum chitotriosidase activity between treatment groups after 6 months (P = .1578) (Table 2).

No relationship was found between total cholesterol, triglycerides, LDL-cholesterol and high-density lipoprotein (HDL)-cholesterol concentrations, and the serum chitotriosidase activity at baseline in either treatment group.

After 6 months of treatment, serum chitotriosidase activity did not show any relationship with total cholesterol triglycerides, LDL-cholesterol, and HDL-cholesterol variations in the group as a whole, and in either treatment group or chitotriosidase genotype group.

DISCUSSION

The allelic frequency of the 24-bp duplication in the chitotriosidase gene that causes deficiency in chitotriosidase activity was 0.26 in the Spanish population of this study. This percentage is very similar to the 0.24 reported in Dutch or the 0.23 reported in Ashkenazi Jewish populations. These data suggest that this mutation is relatively ancient in the evolution, with similar presentation in different ethnic groups, and that a significant 6% and 40% of the general population are homozygous and heterozygous, respectively, for this chitotriosidase activity deficiency.

According to the relationship genotype and phenotype, chitotriosidase deficiency in humans appears as an autosomal incompletely dominant disorder, with no activity in homozygous subjects for the defective allele and approximately half-normal activities in heterozygous subjects (Table 1).

We could not show any modification of the serum chitotriosidase activity after 6 months of lipid-lowering treatment with either atorvastatin or bezafibrate. Chitotriosidase activities remained constant throughout the study, suggesting that the important LDL-cholesterol and triglyceride reduction obtained with both drugs (35% and 25% for atorvastatin and 10% and 35% for bezafibrate, respectively) (Table 2), did not modify the macrophage chitotriosidase expression in these subjects, at least in a sufficient level to be detected in serum by the current measurements of this enzyme activity.

Our results do not support the idea that chitotriosidase activity could be used as a biologic marker of atherosclerotic plaque modification related to hypolipidemic treatment, at least after only several months of treatment.

The mechanisms underlying the induction of chitotriosidase expression in macrophages and the reason why it is over-expressed in the arteriosclerotic vascular lesion are unknown. We speculated that lipid accumulation inside the macrophage related to hyperlipidemia could be involved in this process; however, plasma lipid correction does not seem to interfere in the chitotriosidase expression level in vivo.

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