Serum Chitotriosidase Activity Is Increased in Subjects With Atherosclerosis Disease

Marta Artieda, Ana Cenarro, Alberto Gañán, Ivonne Jericó, Carmen Gonzalvo, Juan M. Casado, Isabel Vitoria, José Puzo, Miguel Pocoví, Fernando Civeira

Objective—This study was undertaken to analyze the relation between serum activity of chitotriosidase enzyme, a protein synthesized exclusively by activated macrophages, and atherosclerotic lesion extent in subjects with atherothrombotic stroke (ATS) and in subjects with ischemic heart disease (IHD).

Methods and Results—We assayed the serum chitotriosidase activity and a common chitotriosidase gene polymorphism that causes deficiency in chitotriosidase activity in 3 Spanish populations, ATS (n=153), IHD (n=124), and control (n=148) subjects. Statistical differences were found in serum chitotriosidase activity between ATS (88.1±4.6 nmol/mL·h, P<0.0001) and IHD subjects (79.0±6.3, P=0.002) versus control group (70.9±5.2). These observed differences were not attributable to a distinct allelic or genotype distribution. The extension of the atherosclerotic lesion in carotids of ATS subjects was measured by duplex sonography. Chitotriosidase activities were 66.9±9.6, 88.7±8.3, and 107.7±11.8 for subjects with carotid stenosis ≤30%, 31% to 60%, and >60%, respectively. Statistical differences were observed between subjects with major and intermediate stenosis grade compared with subjects with minor stenosis, P=0.005 and P=0.016, respectively.

Conclusions—Serum chitotriosidase activity is significantly increased in individuals suffering from atherosclerosis disease and is related to the severity of the atherosclerotic lesion, suggesting a possible role as atherosclerotic extent marker.

(Arterioscler Thromb Vasc Biol. 2003;23:1645-1652.)

Key Words: chitotriosidase ■ macrophage ■ atherothrombotic stroke ■ ischemic heart disease ■ atherosclerosis

Atherosclerosis is a chronic arterial disease with 2 life-threatening complications, myocardial and cerebral infarcts. The long-lasting process of atherogenesis involves important modifications in the cellular and extracellular matrix and lipid components of the arterial wall, resulting in intimal thickening, vessel lumen narrowing, and increased susceptibility to thrombosis.1–3 Among them, activated macrophages inside the atherosclerotic lesion play an important role in the evolution of the vascular plaque. The formation of lipid-laden foam cells from macrophages represents a landmark for atherosclerosis.4 5 The development of foam cells is mainly attributable to overloading of lipids, particularly cholesterol and cholesterol ester, into the cells through a scavenger receptor–mediated process.6 This lipid accumulation promotes the expression of different genes in the macrophage that could influence the inflammatory process that occurs in the atherogenesis.2 A better understanding of atherogenesis requires a more precise characterization of the proteins secreted in the vessel wall by macrophages that are involved in this pathological process.

Chitotriosidase, one of the most quantitative proteins secreted by activated macrophages, is a human chitinase member of family 18 glycosyl hydrolases.7–9 It is synthesized as a 50-kDa protein containing a 39-kDa N-terminal catalytic domain, a hinge region, and a C-terminal chitin-binding domain. It is predominantly secreted but in part processed into a 39-kDa form that accumulates in lysosomes. In the bloodstream, the secretory 50-kDa chitotriosidase occurs predominantly, whereas in tissues, the 39-kDa form is also abundant.10 A common chitotriosidase gene polymorphism leads to a null allele and therefore a defective protein. The nature of this common deficiency in chitotriosidase activity is a 24-base pair (bp) duplication in exon 10 of the chitotriosidase gene that results in activation of a cryptic 3′ splice site, generating a mRNA with an in-frame deletion of 87 nucleotides, encoding a protein that lacks an internal stretch of 29 amino acids.11 This genetic variation is responsible for the recessive inherited deficiency in chitotriosidase activity and is found in individuals from various ethnic origins. In white populations, 30% to 40% of individuals are carriers of this
abnormal chitotriosidase allele and approximately 6% are homozygous.12,13

Chitotriosidase 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’s disease.12,14 Recently, Boot et al15 have 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 human atherosclerotic vessel wall.

The aim of this study was to determine whether serum chitotriosidase activity is increased in individuals experiencing an ischemic stroke of atherothrombotic etiology and in subjects with ischemic heart disease and, in that way, if chitotriosidase activity could be related to the extension of atherosclerosis.

To evaluate our hypothesis, we analyzed the serum chitotriosidase activity and the described chitotriosidase gene polymorphism in a group of subjects with ischemic stroke of atherothrombotic origin, in a group of subjects with ischemic heart disease, and in a group of control subjects.

Methods

**Study Subjects**

The atherothrombotic stroke (ATS) group consisted of 153 nonrelated Spanish subjects younger than 71 years of age with an ischemic stroke defined as a brunt onset of a focal neurological deficit attributable to a cerebral infarct by occlusion or stenosis of atherosclerotic etiology in an intracranial or extracranial artery. Inclusion criteria were based on TOAST criteria.16 Exclusion criteria were cardioembolic, lacunar and undetermined strokes, and intracerebral hemorrhage.

The ischemic heart disease (IHD) group consisted of 124 nonrelated Spanish subjects with stabilized unstable angina. Inclusion criterion was the diagnosis of ischemic heart disease by coronary arteriography with at least 1 of the following conditions in the angiographic scores: vessel score ≥1, stenosis score ≥4, and extent score ≥12. Exclusion criteria were acute myocardial infarction, coronary bypass surgery, or coronary angioplasty. A group of 148 nonrelated Spanish subjects with normal lipid profile and without symptomatic atherosclerosis disease was also included in the study and considered the control group.

The study was approved by the ethic committee of the Hospital Universitario Miguel Servet, and all subjects gave written informed consent.

**Samples**

Venous blood samples were collected into tubes containing Na2EDTA (to obtain genomic DNA) and into tubes containing SST clot activating gel (to obtain serum) after a 12-hour fast. The serum samples tubes were allowed to clot before centrifugation. After centrifugation at 4°C for 15 minutes at 3500 rpm, serum was aliquoted and immediately frozen at −80°C. Genomic DNA was isolated from peripheral blood cells using the salting-out method.17 DNA was quantified and diluted to a final concentration of 100 ng/μL to be used in polymerase chain reaction (PCR) analysis. Samples were collected within 48 hours after the clinical onset of the stroke (ATS group) or at the time of programmed coronary arteriography out of the acute event (IHD group).

**Quantification of Lipids and Lipoproteins**

Total serum cholesterol and triglyceride levels were quantified enzymatically with a Beckman Synchron CX7 analyzer (Boehringer Mannheim). HDL cholesterol (HDL-C) was measured after precipitation of apolipoprotein B–containing lipoproteins with Mg2+-phosphotungstate (Boehringer Mannheim). LDL cholesterol (LDL-C) was calculated by the Friedewald formula.18 Lipoprotein(a) [Lp(a)] was determined by kinetic immunonephelometry with polyclonal antibodies (Beckman).19

**Chitotriosidase Enzyme Assay**

Chitotriosidase enzyme assay was based on the method described by Hollak et al20 with minor modifications. Briefly, chitotriosidase activity was determined by incubating 10 μL of serum with 200 μL of 22 μmol/L fluorogenic substrate 4-methylumbelliferyl-β-D-N,N′,N′′-triacyctethylchitotrioside (Sigma) in McIlvain buffer (100 mMol/L citric acid and 200 mMol/L sodium phosphate, pH 5.2) for 15 minutes at 37°C. The reaction was stopped with 2 mL of 0.3 mol/L glycine-NaOH buffer (pH 10.6) by mixing at room temperature. The substrate hydrolysis by chitotriosidase produces the fluorescent molecule 4-methylumbellifere, which was quantified with a fluorometer (Kontron Instruments), excitation at 366 nm and emission at 446 nm, and compared with a standard 4-methylumbellifere calibration curve. Chitotriosidase activity was expressed as nanomoles of substrate hydrolyzed per hour per milliliter of incubated serum. Serum chitotriosidase activity was measured by duplication and coefficient of variation was less than 5% in all cases.

**DNA Analysis**

Determination of the 24-bp duplication in the chitotriosidase gene was performed by PCR followed by electrophoresis of the amplified fragments. PCR reaction was performed using Taq DNA polymerase (Gibco BRL), genomic DNA as template, and the oligonucleotides previously described.11 Genomic DNA was subjected to 30 cycles of denaturation at 95°C for 1 minute, annealing at 48°C for 50 seconds, and extension at 72°C for 50 seconds, followed by a final extension of 10 minutes at 72°C. Fragments of 75 and 99 bp were amplified from wild-type and defective allele, respectively. Amplified DNA fragments were electrophoresed on a 3% NuSieve agarose gel (BioWhittaker Molecular Applications) and visualized by staining with ethidium bromide.

**Assessment of the Atherosclerotic Lesion Extent**

**ATS Group**

Extension of atherosclerotic lesion was measured by duplex sonography combining continuous-wave Doppler and B-mode imaging to evaluate the stenosis degree and the plaque morphology in common and internal carotids.20,21 The extent of carotid atherosclerosis was expressed as stenosis grade in a scale 0% to 100%.

**IHD Group**

Extension of coronary atherosclerosis was evaluated in a blinded manner from results of coronary arteriographies with the following 3 different scores: (1) vessel score, the number of major vessels with significant coronary stenosis according to the BARI protocol;22 (2) stenosis score, the addition of stenosis in 8 different proximal segments (stenosis <50% = 1, 50% to 74% = 2, 75% to 99% = 3 and total occlusion = 4)23–25; and (3) extent score, the addition of segment longitudinal extension of all coronary lesions within the 8 proximal segments.26,27

**High-Sensitivity C-Reactive Protein Assay**

Serum concentrations of high-sensitivity C-reactive protein (hsCRP) were measured by kinetic immunonephelometry (Beckman Coulter Image Immunochemistry System).28 hsCRP was expressed as milligram per liter.

**Statistical Methods**

Most of the statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS Inc) version 6.1.3. A χ2 analysis was performed to confirm that the studied polymorphism in the 3 groups was in Hardy-Weinberg equilibrium.
TABLE 1. Anthropometric and Lipid Data of the Subjects Included in This Study

<table>
<thead>
<tr>
<th>Groups</th>
<th>ATS</th>
<th>IHD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.7±6.8</td>
<td>55.2±7.3</td>
<td>59.0±10.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.0±3.9</td>
<td>28.1±3.9</td>
<td>26.8±3.9</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.04±1.10</td>
<td>6.05±1.04</td>
<td>6.06±1.13</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.66±0.72</td>
<td>1.78±0.71</td>
<td>1.41±0.66</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.26±0.99</td>
<td>4.42±0.90</td>
<td>4.32±0.97</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.01±0.23</td>
<td>0.82±0.24</td>
<td>1.00±0.31</td>
</tr>
<tr>
<td>Lp(a), µmol/L†</td>
<td>1.83±1.96</td>
<td>1.39±1.59</td>
<td>1.05±1.19</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*ANOVA one factor adjusted for sex, age, and BMI.
†Analysis with the Mann-Whitney nonparametric test.

Results

Description of Subjects

Anthropometric and lipid data of the ATS, IHD, and control subjects included in this study are shown in Table 1. There were statistical differences between ATS and control groups in relation to age, total cholesterol, triglycerides, LDL-C, HDL-C, and Lp(a); however, no statistical differences concerning the BMI were found. Triglycerides, Lp(a), and BMI were significantly higher in the IHD group than in control group; age was significantly lower, and no statistical differences were observed in total cholesterol, LDL-C, and HDL-C. Total cholesterol and LDL-C were significantly lower in ATS versus control subjects. This probably reflects the significant decrease of total cholesterol and LDL-C during the first 48 hours after an acute vascular event and the lack of significant correlation between ischemic stroke and serum cholesterol levels. To analyze whether these serum cholesterol differences could be related to the measured chitotriosidase activities, we calculated the correlation coefficients between total cholesterol and chitotriosidase activity for each group. There was no significant correlation between these variables.

Chitotriosidase Gene Polymorphism

The 24-bp duplication polymorphism in exon 10 of the chitotriosidase gene was determined by PCR amplification, as shown in Figure 1. The resulting genotypic and allelic frequencies are shown in Table 2. Frequencies of defective allele were 0.24 (CI 95%, 0.19 to 0.29), 0.20 (CI 95%, 0.15 to 0.25), and 0.22 (CI 95%, 0.17 to 0.27) in the ATS, IHD, and control groups, respectively. There were no differences in the allelic distributions between subjects in ATS and IHD groups versus control group. The genotype distributions were in Hardy-Weinberg equilibrium, and there were no differences in these genotype distributions between subjects in ATS and IHD groups versus control group. No association between the polymorphism and plasma total cholesterol, LDL-C, HDL-C, triglycerides, and Lp(a) levels was observed when all studied groups were analyzed as a whole or for each group separately (data not shown).

Chitotriosidase Activity

Backward linear regression analysis showed a positive correlation between chitotriosidase activity and age (R=0.305, P<0.0001). Because serum chitotriosidase activity correlated with age, comparisons were carried out adjusting for age. In Figure 2, log serum chitotriosidase activities are presented as box whisker plots for each of the 3 studied groups clustered by chitotriosidase genotype. Chitotriosidase activity in the whole ATS group was 88.1±4.6 nmol/mL · h (mean±SE), in
IHD group was 79.0 ± 6.3 nmol/mL · h, and in control group was 70.9 ± 5.2 nmol/mL · h. Differences were statistically significant between ATS versus control subjects (P = 0.0001) and between IHD versus control groups (P = 0.020). Chitotriosidase activities of heterozygous defective allele (ND) subjects were 65.5 ± 4.4, 43.8 ± 4.8, and 35.5 ± 4.4 nmol/mL · h for ATS, IHD, and control groups, respectively. The differences between ATS and control groups (P < 0.0001) and between IHD and control groups (P = 0.023) were statistically significant. In each studied group, serum chitotriosidase activity was significantly higher in homozygous NN subjects than in heterozygous ND subjects (P < 0.0001).

**Chitotriosidase Activity Variability**

To identify predictors for chitotriosidase activity variability, a multiple backward linear regression analysis was performed. The dependent variable was log chitotriosidase activity, and the variables LDL-C, HDL-C, TG, Lp(a), chitotriosidase genotype, age, sex, BMI, and group were included as independent variables. The independent variable group was separated in 2 subgroups in this analysis, subjects with atherosclerosis disease (ATS and IHD groups) and subjects without atherosclerosis disease (control group). Multiple linear regression analysis displayed a correlation (multiple R = 0.611, adjusted R² = 0.366) between log chitotriosidase activity and the independent variables chitotriosidase genotype (NN genotype, B = 0.343 ± 0.030, P < 0.0001; ND genotype, B = −0.343 ± 0.030, P < 0.0001), age (B = 0.011 ± 0.002, P < 0.0001), and group (B = 0.138 ± 0.030, P < 0.0001). B represents the regression coefficient that is expressed as mean ± SD. The variables LDL-C, HDL-C, TG, Lp(a), sex, and BMI did not correlate with chitotriosidase activity.

**Predictive Value of Chitotriosidase Activity Measurements**

To evaluate the value of chitotriosidase activity measurements in the diagnosis of atherosclerosis disease, we calculated the sensitivity, specificity, accuracy, and positive and negative predictive values of chitotriosidase activity measurements for ATS (NN) subjects, ATS (ND) subjects, IHD (NN) subjects, and IHD (ND) subjects versus control group, respectively, as shown in Table 3. These values were calculated at various chitotriosidase activity cutoff levels, the 10th, 25th, 50th, and 75th percentiles.

**TABLE 2. Chitotriosidase Genotype Distribution and Allelic Frequency**

<table>
<thead>
<tr>
<th>Groups</th>
<th>ATS</th>
<th>IHD</th>
<th>Control</th>
<th>ATS vs Control Group, P*</th>
<th>IHD vs Control Group, P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous normal allele, (NN) %</td>
<td>58.2 (89)</td>
<td>65.3 (81)</td>
<td>61.5 (91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygous defective allele, (ND) %</td>
<td>35.9 (55)</td>
<td>29.0 (36)</td>
<td>32.4 (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous defective allele, (DD) %</td>
<td>5.9 (9)</td>
<td>5.7 (7)</td>
<td>6.1 (9)</td>
<td>0.813</td>
<td>0.806</td>
</tr>
<tr>
<td>Wild type allele (N)</td>
<td>0.76 (233)</td>
<td>0.80 (198)</td>
<td>0.78 (230)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defective allele (D)</td>
<td>0.24 (73)</td>
<td>0.20 (50)</td>
<td>0.22 (66)</td>
<td>0.650</td>
<td>0.545</td>
</tr>
</tbody>
</table>

In parenthesis are indicated number of subjects (genotype frequency rows) and number of alleles (allelic frequency rows).

*χ² analysis.
Stenosis and Chitotriosidase Activity

To assess the relationship between chitotriosidase activity and the arterial intima-media thickness, we analyzed the carotid stenosis data of the ATS subjects proved by duplex sonography. The ATS group presented a carotid stenosis of 55.4 ± 20.4% (mean ± SD). Chitotriosidase activities for 3 subgroups with different stenosis grade were as follows: 66.9 ± 9.5 nmol/mL · h for stenosis ≤ 30%, 88.7 ± 8.3 nmol/mL · h for stenosis 31% to 60%, and 107.2 ± 11.8 nmol/mL · h for stenosis > 60%. Statistical differences were found between subjects with stenosis of 31% to 60% and subjects with stenosis > 60% compared with subjects with stenosis ≤ 30%, *P = 0.016 and **P = 0.005, respectively. To rule out the possibility that chitotriosidase activity differences observed were attributable to a distinct genotype distribution, we also performed this analysis by genotype, as shown in Figure 3. Homozygous NN and heterozygous ND subjects showed increased levels of chitotriosidase activity with larger stenosis grade, as follows: NN subjects, 87.0 ± 14.4, 112.6 ± 9.7, and 138.2 ± 16.5 nmol/mL · h; ND subjects, 49.5 ± 8.4, 68.7 ± 8.5, and 82.1 ± 10.1 nmol/mL · h for stenosis ≤ 30%, 31% to 60%, and > 60%, respectively.

The same analysis was carried out with the angiographic scores of the IHD subjects. The IHD group presented a vessel score of 1.03 ± 0.84, a stenosis score of 9.47 ± 4.10, and an extent score of 42.92 ± 18.70 (mean ± SD). In this case, when chitotriosidase activity was compared with the angiographic scores, no significant differences were found.

### Table 3: Sensitivity, Specificity, Accuracy and Predictive Value of Chitotriosidase Activity in ATS and IHD Groups

<table>
<thead>
<tr>
<th>Group (Chitotriosidase Genotype)</th>
<th>Chitotriosidase Activity Cutoff Level, percentile</th>
<th>Chitotriosidase Activity nmol/mL · h</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
<th>Accuracy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATS (NN)</td>
<td>10</td>
<td>54.2</td>
<td>91 (85–97)</td>
<td>32 (22–41)</td>
<td>56 (48–64)</td>
<td>78 (65–92)</td>
<td>61 (54–68)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>65.8</td>
<td>75 (66–84)</td>
<td>44 (34–54)</td>
<td>56 (47–65)</td>
<td>65 (53–76)</td>
<td>59 (52–66)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100.5</td>
<td>50 (40–60)</td>
<td>64 (54–74)</td>
<td>57 (46–68)</td>
<td>57 (47–66)</td>
<td>57 (50–64)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>130.7</td>
<td>25 (16–34)</td>
<td>71 (62–81)</td>
<td>46 (32–60)</td>
<td>50 (41–58)</td>
<td>49 (41–56)</td>
</tr>
<tr>
<td>ATS (ND)</td>
<td>10</td>
<td>29.8</td>
<td>91 (83–99)</td>
<td>50 (36–64)</td>
<td>68 (57–76)</td>
<td>83 (69–97)</td>
<td>72 (63–81)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>37.7</td>
<td>76 (65–88)</td>
<td>63 (49–76)</td>
<td>70 (58–82)</td>
<td>70 (56–83)</td>
<td>70 (61–79)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>62.1</td>
<td>51 (38–64)</td>
<td>88 (78–97)</td>
<td>82 (70–95)</td>
<td>61 (49–72)</td>
<td>68 (59–77)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>85.2</td>
<td>25 (14–37)</td>
<td>96 (90–101)</td>
<td>88 (71–104)</td>
<td>53 (42–63)</td>
<td>58 (49–68)</td>
</tr>
<tr>
<td>IHD (NN)</td>
<td>10</td>
<td>43.0</td>
<td>89 (82–96)</td>
<td>22 (13–30)</td>
<td>50 (42–59)</td>
<td>69 (52–86)</td>
<td>53 (46–61)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>59.0</td>
<td>74 (65–84)</td>
<td>38 (28–48)</td>
<td>54 (45–63)</td>
<td>57 (45–70)</td>
<td>55 (48–63)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>81.5</td>
<td>51 (40–62)</td>
<td>52 (41–62)</td>
<td>48 (38–59)</td>
<td>54 (44–64)</td>
<td>51 (44–59)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>113.0</td>
<td>25 (15–34)</td>
<td>66 (56–76)</td>
<td>39 (26–53)</td>
<td>50 (41–58)</td>
<td>47 (39–54)</td>
</tr>
<tr>
<td>IHD (ND)</td>
<td>10</td>
<td>15.5</td>
<td>89 (79–99)</td>
<td>19 (8–30)</td>
<td>45 (33–57)</td>
<td>69 (44–94)</td>
<td>49 (38–59)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.0</td>
<td>78 (64–91)</td>
<td>46 (32–60)</td>
<td>52 (39–65)</td>
<td>73 (58–89)</td>
<td>60 (49–70)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>39.3</td>
<td>50 (34–66)</td>
<td>69 (56–82)</td>
<td>55 (38–72)</td>
<td>65 (52–78)</td>
<td>61 (50–71)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>56.3</td>
<td>25 (11–39)</td>
<td>88 (78–97)</td>
<td>60 (35–85)</td>
<td>61 (49–72)</td>
<td>61 (50–71)</td>
</tr>
</tbody>
</table>

In parenthesis are indicated the 95% CIs.
Protein secretion.7 In this study, serum chitotriosidase activity has been assayed using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside to show full inhibition of its enzymatic activity. Because they found a correlation between the amount of chitotriosidase activity and the high-sensitivity C-reactive protein, a well-known inflammatory protein. For this purpose, we measured the high-sensitivity C-reactive protein (hsCRP) in 138 control subjects, the values being 0.34±0.06 mg/L (mean±SE). Moreover, we measured the chitotriosidase activity and hsCRP in 43 patients of lysosomal Gaucher’s disease, genetic and biochemically diagnosed,11 in which chitotriosidase activity is a biochemical marker, because it is over 2 orders of magnitude compared with healthy control individuals. hsCRP in these subjects was 0.42±0.13 mg/L. Chitotriosidase activity was 170.70-fold increased compared with control subjects, 12107.8±1776.2 versus 70.9±5.2 nmol/mL · h (mean±SE). There were statistical differences in chitotriosidase activity between Gaucher versus control subjects (P<0.0001), but no statistical differences in hsCRP values were found. Backward linear regression analysis showed absence of correlation between chitotriosidase activity and hsCRP in both groups.

Discussion

The enzyme chitotriosidase, the human analogue of chitinases from nonvertebrate species, is exclusively produced in humans by activated macrophages.10 The production of chitotriosidase by macrophages is highly variable depending on different circumstances, and in some occasions it is the main protein secreted, representing approximately 1% of the total protein secretion.7 In this study, serum chitotriosidase activity has been assayed using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside by duplication, being the coefficient of variation less than 5%, which would indicate that this assay has a good reproducibility. Furthermore, the method used is highly specific, as was demonstrated by Boot et al15 by preincubating extracts from apparently normal and atherosclerotic tissue with a polyclonal antibody directed against chitotriosidase, which showed full inhibition of its enzymatic activity. Because they found a correlation between the amount of chitotriosidase mRNA in atherosclerotic tissue and chitotriosidase activity, this would suggest that measuring the enzyme activity would provide the gene expression status.15

The allelic frequencies of the 24-bp duplication in the chitotriosidase gene that causes deficiency in chitotriosidase activity in the ATS, IHD, and control groups were very similar to those reported in Dutch and Ashkenazi Jewish populations.11 These data suggest that this duplication seems to be relatively ancient in the evolution. Because there were no differences in the allelic and genotype distributions between the studied groups, we can rule out the possibility that chitotriosidase activity differences observed between groups were attributable to distinct genotype distributions, and, therefore, we can rule out a pathogenetic role of chitotriosidase gene polymorphism in atherosclerosis disease. According to the relationship between genotype and activity observed in this study, chitotriosidase activity seems to be inherited as an autosomal incompletely dominant trait, with no chitotriosidase activity in homozygous DD subjects and approximately half NN activities in heterozygous ND subjects.

Enzyme chitotriosidase can be considered an inflammatory protein because it is only secreted by activated macrophages, but its production happens after at least 1 week of cell culture and increases with time; therefore, it does not behave as an acute reactive protein but rather as a chronic inflammatory marker.7 We investigated the relation of chitotriosidase activity with high-sensitivity C-reactive protein, a well-known marker of overall systemic inflammation, in control subjects and in patients with Gaucher’s disease. The high increase of chitotriosidase activity in Gaucher’s disease is secondary to a well-established primary defect, a deficiency of the lysosomal hydrolase β-glucocerebrosidase attributable to mutations in its gene that produce macrophage activation by its intracellular glucosylceramide accumulation. Only chitotriosidase activity was highly increased in patients with Gaucher’s disease, hsCRP showed the same levels as control subjects, and there was no correlation between chitotriosidase activity and hsCRP in both groups. Therefore, these inflammatory markers seem to be regulated by different mechanisms. In accordance with this, it has been reported that chitotriosidase activity is related to intracellular lipid accumulation in Gaucher’s disease12,33,34 and does not modify after 6 months of hypolipidemic treatment,35 whereas hsCRP decreases by 29% in the same subjects with mixed hyperlipemia.36 Previous studies have demonstrated that the macrophages within atherosclerotic vascular plaques produce high amounts of chitotriosidase,15 which led us to hypothesize that serum chitotriosidase activity could be related to the amount of lipid-loaded macrophages in the atherosclerotic arterial wall, as it does occur in Gaucher’s disease. The results presented in this work confirm our hypothesis, because serum chitotriosidase activity in ATS and IHD patients was higher than in control subjects. One important aspect of our study was the precise selection of the patients included to ensure that all had atherosclerosis disease. Especially in the stroke group, where the etiology may be very heterogeneous, we selected only those subjects with specific criteria of atherothrombotic cerebrovascular disease, excluding other possible etiologies.
Moreover, the atherosclerotic lesion extent was quantified in all selected subjects from ATS and IHD groups.

As described above, ATS and IHD groups had significantly higher chitotriosidase activities than the control group, and ATS subjects had higher chitotriosidase activity than IHD subjects. The latter observation probably reflects the fact that subjects with atherothrombotic cerebrovascular disease have an atherosclerosis process more widespread than subjects with ischemic heart disease, whose atherosclerosis is localized more specifically in the coronary vessels. Moreover, our results have shown that chitotriosidase activity was related to carotid stenosis in the ATS group, which is in accordance with a situation of more widespread atherosclerosis. According to this hypothesis, previous reported series and clinical trials have demonstrated that patients with cerebrovascular disease are more likely to die in follow-up from cardiovascular than from cerebrovascular causes.

In this study, we have found an age-dependent increase in serum chitotriosidase activity. A similar effect of age on chitotriosidase activity has been previously described in subjects with different lysosomal disorders and in the general population. This age-dependent increase in chitotriosidase activity could be explained by the ongoing accumulation of lipid-laden macrophages during the gradual progression of atherosclerosis in relation to age.

Laboratory and prospective clinical studies have recently reinforced the inflammatory theory proposed by Ross, who stated that each step of the molecular and cellular responses leading to atherosclerosis is an inflammatory process. At present, it is considered that inflammation plays both a central role in the beginning of atherosclerosis process and in the mechanism underlying the development and progression of the atherosclerosis complications, plaque rupture and subsequent thrombosis. Continued inflammation results in increased numbers of macrophages and lymphocytes, both markers of ongoing inflammation, emigrating from the blood into the lesion. Macrophages are present in all phases of atherogenesis and are markers of unstable atherosclerotic plaques. Macrophage-rich areas are more frequently found in patients with unstable angina than in patients with stable angina. Our results would suggest that macrophage accumulation localized in supra-aortic and coronary vessels is associated with increased serum chitotriosidase activity. If true, chitotriosidase activity could reflect the state of activation of macrophages, possibly within atherosclerotic lesions.

The exact mechanism underlying the induction of chitotriosidase expression in macrophages in unknown. We can only speculate on the physiological role of chitotriosidase in atherogenesis, because no human endogenous chitin-like substances are known at present. Recently, Boot et al reported the identification of a second mammalian chitinase named acidic mammalian chitinase in the gastrointestinal tract and lung. It is possible that the acidic mammalian chitinase could play a role in digestion and/or defense because of its expression tissue distribution. Meanwhile, the chitotriosidase could be involved in the tissue-remodelling processes that take place during atherogenesis.

Chitotriosidase activity measurement in clinical practice has several important limitations. First, it is necessary to adjust serum activity with chitotriosidase genotype because of the high frequency of the defective allele in the general population. Second, there is a large range of serum chitotriosidase activity values in control subjects, even sharing the same genotype, and an overlap exists between serum chitotriosidase activity values from control and affected subjects. For this reason, the sensitivity and specificity of chitotriosidase activity measurements at the analyzed cutoff levels have values with high sensitivity but low specificity or with high specificity but low sensitivity. Therefore, the accuracy has values below 72% in both ATS and IHD groups and in both NN and ND subjects. Third, little is known about physiological role of chitotriosidase and the mechanisms that could modify its activity in humans, and they could be relevant in clinical practice. For these reasons, chitotriosidase activity measurements in the diagnosis of atherosclerosis disease have relatively weak predictive value as a marker of atherosclerotic lesion.

Three major conclusions can be made from our results, with the limitations described above. High serum chitotriosidase activity in patients with atherosclerosis demonstrates in vivo the presence of activated macrophages in such subjects. Serum chitotriosidase activity is related to the severity of the atherosclerotic lesions, suggesting a possible role as a marker of atherosclerotic extension. Finally, this increase of serum chitotriosidase activity demonstrates that it is feasible to measure functional aspects of macrophages from blood samples of patients with atherosclerosis.

Acknowledgments

This work was supported by grants from Fondo de Investigación Sanitaria (FIS 00/0952), Dupuitación General de Aragón (DGA P016/99-BM), and Ministerio de Ciencia y Tecnología (SAF 2001-2466-C05). M.A. is a recipient of a fellowship from Fondo de Investigación Sanitaria, and A.G. has a FPI fellowship from Ministerio de Educación y Ciencia.

References


Serum Chitotriosidase Activity Is Increased in Subjects With Atherosclerosis Disease
Marta Artieda, Ana Cenarro, Alberto Gañán, Ivonne Jericó, Carmen Gonzalvo, Juan M. Casado, Isabel Vitoria, José Puzo, Miguel Pocoví and Fernando Civeira

Arterioscler Thromb Vasc Biol. 2003;23:1645-1652; originally published online July 31, 2003; doi: 10.1161/01.ATV.0000089329.09061.07
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/9/1645

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/