(TTA)n Polymorphism in 3-Hydroxy-3-Methylglutaryl-Coenzyme A and Response to Atorvastatin in Coronary Artery Disease Patients

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Abstract: 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors have been used clinically for lowering total and low-density lipoprotein cholesterol. Interindividual pharmacological differences observed with this treatment have been attributed to genetic differences. The aim of this study was to assess the association in the low-density lipoprotein cholesterol reduction by atorvastatin and (TTA)n polymorphism in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene in patients with coronary artery disease. Changes in total cholesterol levels, triglycerides, high-sensitivity C-reactive protein and free F_2-isoprostanes were also evaluated. In an open study, patients received 40 mg atorvastatin daily for 8 weeks. Genotyping was done through polymerase chain reaction. The genotype distribution of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (TTA)n polymorphism was: >10/>10 in 22 out of 64 patients (34%), >10/10 in 14 out of 64 patients (22%) and 10/10 in 28 out of 64 patients (44%). The reduction of low-density lipoprotein cholesterol levels by atorvastatin was not different between allelic variants (TTA)n repeat polymorphism. Reductions in high-sensitivity C-reactive protein were observed in atorvastatin-treated patients with alleles >10/>10 and 10/10. Free F_2-isoprostanes and total cholesterol were also significantly lower after treatment for all alleles, irrespective of type of polymorphism. In conclusion, the changes induced by atorvastatin treatment on low-density lipoprotein cholesterol, total cholesterol, triglycerides, high-sensitivity C-reactive protein and free F_2-isoprostane concentrations were not related to the presence of 3-hydroxy-3-methylglutaryl-coenzyme A reductase polymorphism (TTA)n.

Therapy with 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, namely, statins, is widely used for the treatment of hypercholesterolaemia, decreasing total and low-density lipoprotein cholesterol. Statin therapy also lowers C-reactive protein levels in a low-density lipoprotein cholesterol-independent manner [1–3]. Statins have antioxidant properties as well [4] and are prescribed for both primary [5–7] and secondary [8–11] prevention of coronary artery disease. However, there is considerable interindividual variation in their hypolipidaemic response [12].

Studies about genetic variations in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene and the relationship with changes in lipid levels with statin therapy are limited [13,14].

Previous studies have indicated that a variable number of tandem repeats polymorphism (TTA)n of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene was related to pathologies, such as coronary artery disease and hypercholesterolaemia [15,16]. (TTA)n polymorphism of 3-hydroxy-3-methylglutaryl-coenzyme A reductase is localized in the intron 2 and there are not data about the expression and/or activity of the enzyme.

Moreover, an almost significant difference between the (TTA)n repeat polymorphism in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene and cholesterol absorption has been reported in response to plant stanol consumption [17].

The aim of this study was to evaluate the role of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (TTA)n repeat polymorphism in connection with the low-density lipoprotein cholesterol lowering effectiveness of atorvastatin in Chilean patients with coronary artery disease. In addition, a secondary goal was to determine the change in total cholesterol levels, triglycerides, high-sensitivity C-reactive protein and free F_2-isoprostanes in the same group of patients.

Materials and Methods

Study design and patient selection. This study included 69 patients with coronary heart disease treated with either coronary artery bypass grafting or angioplasty, from the Cardiovascular Department, Hospital Clinic, University of Chile. It was an open study, in which patients received 40 mg atorvastatin daily for 8 weeks. During the first visit, anamnesis and physical examination were performed. In addition, a low-calorie diet was recommended. During follow-up, hepatic enzymes and creatinine kinase were measured as statin safety indicator. Ethical approval of the study was obtained from the Ethical Clinical Board of the Hospital Clinic, University of Chile (Santiago, Chile) and all patients gave written consent before participation in the study.

Male and female patients were included, over 18 years of age with angiographically documented coronary artery disease, baseline levels of low-density lipoprotein cholesterol ranging from 100 to 220 mg/dl, triglyceride levels under 400 mg/dl, not having used statins for 2 months prior to enrollment and without contraindication to these drugs. The patients were excluded in case of an acute coronary

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Table 1.

Baseline characteristics of patients with coronary artery disease distributed according to the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (TTA)n repeat polymorphism.

	(TTA) >10/>10 (n = 22)	(TTA) >10/10 (n = 14)	(TTA) 10/10 (n = 28)
Age (years)	64 ± 8	65 ± 9	66 ± 8
Men	16 (73%)	12 (86%)	23 (82%)
Body mass index (kg/m^2)	29 ± 5	26 ± 4	28 ± 3
Chronic therapy			
ACE inhibitors	13 (59%)	9 (64%)	14 (50%)
Angiotensin receptorantagonists	1 (5%)	1 (7%)	5 (18%)
β-Blockers	15 (68%)	7 (50%)	17 (61%)
Diuretics	5 (23%)	2 (14%)	11 (39%)
Calcium antagonists	5 (23%)	2 (14%)	2 (7%)
Aspirin	11 (50%)	13 (93%)	26 (93%)
Laboratory			
Total cholesterol (mg/dl)	207 ± 29	229 ± 39	213 ± 37
LDL cholesterol (mg/dl)	134 ± 23	141 ± 30	137 ± 34
HDL cholesterol (mg/dl)	41 ± 9	49 ± 17	41 ± 10
Triglycerides (mg/dl)	158 ± 63	183 ± 69	175 ± 86
hsCRP (mg/l) (range)	2.6 (0.4–20.3)	1.2 (0.1–6.9)	2.1 (0.1–14.1)
F_2-isoprostane (pg/ml)	44.2 ± 20	53.1 ± 19	48.9 ± 23

LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; PTCA, previous percutaneous coronary angioplasty.
Values are: means ± standard deviation (S.D.), numbers of patients (percentages), or medians (ranges).
Values not significant between groups.

syndrome and coronary revascularization procedure within 90 days, or in case of acute or long-term inflammatory process or diabetes mellitus. Additionally, patients with active liver disease, determined by hepatic transaminase greater than twice the normal upper limit, serum creatinine kinase levels above three times the normal upper limit and serum creatinine above 2.0 mg/dl were also excluded. Pregnancy tests were not performed, because all female patients were post-menopausal.

Enrollment of patients was designed to provide 80% power significance (α) level of 0.05 to detect a 20% difference in low-density lipoprotein cholesterol level.

Laboratory testing. Venous blood was drawn on the first and the final visit, for serum lipid levels, high-sensitivity C-reactive protein and free F_2-isoprostanes. The high-sensitivity C-reactive protein levels were assessed through an assay from Dade-Behring (Deerfield, IL, USA) [18], and free F_2-isoprostanes were determined as described by Pradelles *et al.* [19]. Serum lipid levels were measured using enzymatic methods. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [20].

Genotyping. Genomic DNA was extracted from a blood sample using standard extraction procedures. Genotyping for the (TTA)n repeat polymorphism within the intron 2 of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene was carried out through a polymerase chain reaction, according to Lalovic *et al.* [21]. Seven different products, ranging from 175 bp to 193 bp, were amplified using 5′-CAGAGTGACACTCTGTCTCC-3′ and 5′-CATGTTCCATCCATGTCTGC-3′ primers, corresponding to 10–16 repeats of the TTA triplet. Therefore, patients were divided into three categories, according to Plat and Mensink [17]. The first group had two alleles with more than 10 TTA repeats (>10/>10), the second group had one allele with more than 10 TTA repeats and the other allele with 10 TTA repeats (<10/10), and the third group had two alleles with 10 TTA repeats (10/10).

Statistical analysis. The genotype frequencies were in Hardy–Weinberg equilibrium, assessed using the chi-square test. Differences in continuous variables were evaluated by Student's test, one-way analysis of variance or the Kruskal–Wallis rank test. Non-parametric testing (Kruskal–Wallis rank test) was used to assess differences in baseline changes. Data were reported as mean and standard deviation (S.D.). For polymerase chain reaction, medians were reported. $P < 0.05$ was considered statistically significant.

Results

Study population.

Sixty-nine patients met all inclusion criteria and five of them were withdrawn (one for angiooedema, another for gastric cancer and three patients lost to follow-up). Sixty-four patients completed the 8-week statin treatment period.

The genotype distribution of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (TTA)n polymorphism was as follows: 22 out of 64 patients (34%) were a >10/>10, 14 out of 64 patients (22%) were a >10/10, and 28 of 64 patients (44%) were a 10/10. The three groups had homogeneous baseline characteristics (table 1).

Changes in low-density lipoprotein cholesterol and total serum cholesterol.

An important finding was the change in the low-density lipoprotein cholesterol by atorvastatin, as compared to baseline (table 2). At the end of 8 weeks, low-density lipoprotein cholesterol levels were reduced significantly, with values of 46%, 46% and 45% in the genotype distribution >10/>10, >10/10 and 10/10, respectively ($P < 0.05$), as can be seen in fig. 1. In addition, total serum cholesterol levels were reduced significantly as compared to basal values, with the reduction of 35%, 32% and 34% for each group, respectively ($P < 0.05$), as shown in fig. 2. Moreover, no differences between groups were obtained in low-density lipoprotein cholesterol and total serum cholesterol reductions (figs 1 and 2).

Table 2.

Changes on cholesterol, triglycerides, high-sensitivity C-reactive protein and free F_2-isoprostane levels after 8 weeks of treatment with atorvastatin according 3-hydroxy-3-methylglutaryl-coenzyme A reductase (TTA)n polymorphism in patients with coronary artery disease.

	(TTA) >10/>10 (n = 22)	(TTA) >10/10 (n = 14)	(TTA) 10/10 (n = 28)
Total cholesterol (mg/dl)	−74 ± 29	−74 ± 28	−73 ± 28
LDL cholesterol (mg/dl)	−63 ± 22	−65 ± 25	−61 ± 24
HDL cholesterol (mg/dl)	−0.7 ± 7	−3 ± 8	0.7 ± 6
Triglycerides (mg/dl)	−53 ± 55	−37 ± 60	−60 ± 71
hsCRP (mg/l), median (range)	−1.2 ± 6.4	−0.3 ± 2.4	−0.9 ± 3.9
	0.8 (−18.2 to 19.0)	0.2 (−2.3 to 6.6)	0.5 (−11.5 to 12.5)
F_2-isoprostane (pg/ml)	−21 ± 18	−23 ± 21	−25 ± 25

LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.
Values are: mean of change ± standard deviation (S.D.). For high-sensitivity C-reactive protein, the median is also displayed (range).
Values are not significant between groups. The values after 8 weeks of treatment compared to the basal values, with the exception of HDL cholesterol, are significant ($P < 0.05$).

Changes in high-sensitivity C-reactive protein and free F_2-isoprostanes.

Atorvastatin-induced changes in high-sensitivity C-reactive protein and free F_2-isoprostanes are shown in table 2. At the end of 8 weeks, high-sensitivity C-reactive protein levels were reduced significantly, compared to baseline, with values of 1.2 mg/l and 0.9 mg/l in the genotype distribution, for patient groups >10/>10 and 10/10, respectively ($P < 0.05$). However, in the >10/10 group, no significant reduction was obtained. In addition, free F_2-isoprostane levels were reduced significantly compared to basal values, with changes of 21, 23 and 25 pg/ml for patient groups >10/>10, >10/10 and 10/10, respectively ($P < 0.05$). Besides, the high-sensitivity C-reactive protein and free F_2-isoprostane levels were not significantly reduced between the groups. All these results are shown in table 2.

Discussion

Although atorvastatin is widely prescribed for the treatment of hypercholesterolaemia, it displays marked interindividual variability in its lipid-lowering effectiveness [12]. Genetic variation in 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the direct target of statin therapy, is surprisingly understudied. In the present study, we assessed the association between low-density lipoprotein cholesterol reduction by atorvastatin and (TTA)n repeat polymorphism in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene in coronary artery disease patients. We also evaluated atorvastatin-induced changes in the levels of total cholesterol, high-sensitivity C-reactive protein, free F_2-isoprostane, triglycerides and their relationship with the (TTA)n polymorphism.

The allele frequency obtained in the present study was different from those observed in the European population [17]. Thus, in this study in normal individuals, the genotype distribution of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (TTA)n polymorphism was 21.4% for the >10/>10, 47.3% for the >10/10 and 31.3% for the 10/10. However, in the study population, this distribution was 34% for the >10/>10, 22% for the >10/10 and 44% for the 10/10. These results can be explained by ethnic differences in the populations.

Previous studies have indicated that a variable number of tandem repeats polymorphism (TTA)n of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene was associated

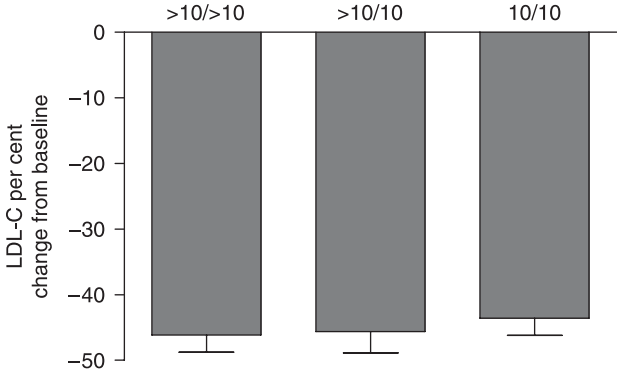

Fig. 1. Per cent change from baseline in low-density lipoprotein (LDL) cholesterol for different (TTA)n polymorphisms at 8 weeks of treatment with atorvastatin.

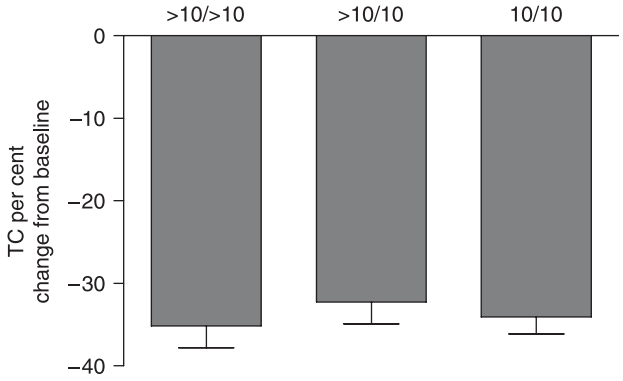

Fig. 2. Per cent change from baseline in total cholesterol (TC) for different (TTA)n polymorphisms at 8 weeks of treatment with atorvastatin.

to pathologies, such as coronary artery disease and hypercholesterolaemia [15,16]. Moreover, it has been reported that this polymorphism has been studied in cholesterol absorption in response to plant stanol consumption [17].

In the present study, the reductions in low-density lipoprotein cholesterol, total cholesterol and triglycerides were not different between allelic variants (TTA)n polymorphism. In relation to another polymorphism in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene, controversial results have been reported. The clinical trial PRINCE (Pravastatin Inflammation/C-reactive protein Evaluation) [13] studied the total cholesterol and low-density lipoprotein cholesterol response associated to two single-nucleotide polymorphisms (SNP), non-coding 3-hydroxy-3-methylglutaryl-coenzyme A reductase variants in tight linkage disequilibrium (SNPs12 and 29). Carriers of the haplotype constituted by these SNPs displayed smaller reductions in low-density lipoprotein cholesterol than non-carriers. However, the ACCESS cohort report [14] was not able to reproduce the precedent results. The triglyceride reduction in the present study is in agreement with the results reported by ACCESS. Aside from PRINCE and ACCESS, no pharmacogenetic studies have examined 3-hydroxy-3-methylglutaryl-coenzyme A reductase and the response to statins. Our findings showed no differences in low-density lipoprotein cholesterol reduction among the three groups of patients with (TTA)n polymorphism.

Pleiotropic effects of statins include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses and stabilization of atherosclerotic plaques [22]. The anti-inflammatory effects have been related to C-reactive protein not only as a marker, but also as an active partaker in this pathological inflammatory process. On the other hand, statins play a role in the oxidative stress through the inhibition of the production of free F_2-isoprostane [23]. The reduction of high-sensitivity C-reactive protein and free F_2-isoprostane induced by atorvastatin, in the present study, reinforce the pleiotropic effects of statins, regardless of the patients' haplotype.

In conclusion, the changes induced by atorvastatin treatment on low-density lipoprotein cholesterol, triglycerides, free F_2-isoprostane, high-sensitivity C-reactive protein and total cholesterol concentrations were not related to the presence of 3-hydroxy-3-methylglutaryl-coenzyme A reductase polymorphism (TTA)n. Data from pharmacogenetic studies are expected to have great impact in the clinical area in the future and will allow the identification of subjects who are likely to get the greatest and the least benefits from a given intervention. For the pharmacogenomic analysis of a specific drug, it seems to be necessary to evaluate several polymorphisms that could be involved in the mechanism of action of this drug.

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