

Intensive Lipid Reduction and Proinflammatory Markers in the MODEST Study

MODEST Çalışmasında Yoğun Lipid Düşürücü Tedavi ve Proinflatuvar Belirteçler

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Abstract

Objective: Statin therapy is well known to reduce inflammatory markers such as tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6), high-sensitivity C-reactive protein (hsCRP). However, whether this relationship is maintained in the setting of targeting very low levels of LDL (<70 mg dl) in patients with type 2 diabetes has not been clearly established.

Materials and Methods: We measured hsCRP, IL-6, and TNF- α in 43 subject enrolled into the multicenter, open-label, crossover prospective study evaluating the effects of lipid-lowering treatment on steroid synthesis in patients with type 2 diabetes (MODEST study). Subjects with diabetes and coronary artery disease were treated with 80 mg of atorvastatin for 12 weeks. The effect of treatment on pro-inflammatory markers was assessed after 12 weeks.

Results: High-dose atorvastatin treatment significantly reduced the plasma levels of IL-6 and hsCRP ($p < 0.05$, $p < 0.001$, respectively), but not of TNF- α ($p = 0.051$).

Conclusion: Atorvastatin treatment targeting very low LDL-cholesterol level reduced the levels of several important inflammatory markers in patients with type 2 diabetes and coronary heart disease. (ClinicalTrials.gov number, NCT00433823). *Türk Jem 2010; 14: 31-4*

Key words: Atorvastatin, hsCRP, interleukin-6, TNF- α , type 2 diabetes

Özet

Amaç: Statin tedavisinin tümör nekroz faktör- α (TNF- α), interleukin 6 (IL-6), yüksek duyarlı C-reaktif protein (hsCRP) gibi inflamatuvar belirteçleri azalttığı iyi bilinmesine rağmen bu ilişkinin çok düşük LDL hedeflenen tip 2 diyabetik hastalarda korunup korunmadığı net olarak gösterilmemiştir.

Gereç ve Yöntemler: MODEST çalışmasında yüksek doz atorvastatin tedavisi alan 43 hasta çalışmaya alınmıştır. Bireylerde tedavi öncesi ve tedavinin 12. haftasında hsCRP, IL-6, and TNF- α düzeyleri değerlendirildi.

Bulgular: Yüksek doz atorvastatin tedavisi ile plazma IL-6 and hsCRP ($p < 0,05$, $p < 0,001$, sırasıyla), değerleri anlamlı olarak düşerken TNF- α düzeylerindeki düşüş sınırdadır ($p = 0,051$).

Sonuç: Çok düşük LDL kolesterol düzeylerini hedefleyen atorvastatin tedavisi tip 2 diabeti ve koroner arter hastalığı olan bireylerde proinflatuvar belirteçler üzerine olumlu etkisini devam ettirmektedir. (ClinicalTrials.gov number, NCT00433823) *Türk Jem 2010; 14: 31-4*

Anahtar kelimeler: Atorvastatin, hs-CRP, interlokin-6, TNF- α , tip 2 diabet

Introduction

Atherosclerotic diseases are the leading causes of death in the developed world (1). Numerous clinical trials of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have demonstrated a significant reduction in cardiovascular events (2). Although the majority of effects could be ascribed to a beneficial effect on the lipid profile, the statins exert beneficial effect, called pleiotropic effect, beyond those predicted by their cholesterol-lowering actions. Pleiotropic effects of statins include improvement in endothelial function, reduction of oxidative stress and inhibition of inflammatory stress (3). Statin therapy is well known to reduce inflammatory markers such as hsCRP, IL-6, TNF- α (4-5). However, whether this relationship is maintained in the setting of targeting very low levels of LDL (<70 mg/dl) in patients with type 2 diabetes has not been clearly established.

A very low level of LDL cholesterol (less than 70 mg per deciliter [1.8 mmol per liter]) has been recommended for patients at high risk of cardiovascular disease. This target was initially proposed as an optional treatment goal in very-high-risk patients with

coronary heart disease (CHD) in the updated Adult Treatment Panel III (NCEP-ATP III) guidelines (6), and it has now been proposed by the American College of Cardiology (ACC) and the American Heart Association (AHA) guidelines in patients with CHD (7). We aimed to investigate whether targeting very low levels of LDL cholesterol obtained with 80 mg of atorvastatin would reduce the inflammatory markers including hsCRP, IL-6, and TNF- α in patient with type 2 diabetes and CHD.

Materials and Methods

Study Population

The participants in this study comprised 43 subjects who were part of the multicenter, open-label, crossover prospective study evaluating the effect of lipid-lowering treatment on steroid synthesis (MODEST study) (8). Patients eligible for inclusion were men and women with type 2 diabetes and those with clinically evident CHD, defined as previous myocardial infarction, previous or current angina with objective evidence of atherosclerotic CHD, or a previous coronary revascularization procedure. The major exclusion criteria were statin hypersensitivity, current statin treatment, liver disease, overt nephropathy (macroalbuminuria [albuminuria >300 mg/day]), pregnancy, breastfeeding, increased creatine kinase levels and postmenopause.

Written informed consent was obtained from all patients and the study was approved by the Ethics Committee.

Study Design

This was a multicenter (n=4), open-label, and prospective study. Since anti-inflammatory effects of statins are seen within weeks we hypothesized a similar time course in patients with type 2 diabetes. As a lipid-lowering treatment, 80 mg of atorvastatin was used for 12 weeks. The patients were evaluated regarding their clinical and laboratory parameters at enrollment and at the end of the 4th, 8th, and 12th weeks of treatment. Efficacy measurements including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and safety measurements of creatine phosphokinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were assessed at enrollment and at each visit. Pro-inflammatory markers including TNF- α , IL-6, hsCRP were evaluated at enrollment and at the end of 12 weeks of treatment. The patients were instructed to take 1 pill per day at bedtime. They were questioned about their compliance with the study drug at every visit. The patients were also assessed regarding the side effects of the study drugs. It was planned that the drug was to be discontinued when CK levels exceeded 10-fold the upper limit of normal, and ALT and AST levels increased to greater than 3-fold the upper limit of normal.

Laboratory Analysis

Samples for serum chemistry and pro-inflammatory markers were analyzed by a central medical research laboratory. Glucose, total cholesterol, triglyceride, AST, and ALT levels were measured using colorimetric enzymatic methods on the Abbott Architect C8000 analyzer and the same manufacturer's reagents. LDL cholesterol concentration was calculated using the Friedewald formula. TNF- α and hsCRP levels were quantified by solid-phase chemiluminescent immunometric assays and IL 6 levels by a solid-phase, enzyme-labeled, chemiluminescent sequential

Table 1. Baseline Characteristics of the Patients

Demographic and Clinical Characteristics	Patients (N=43)
Sex- no (%)	
Male	23 (53)
Female	20 (47)
Age (years)*	45 \pm 10
Systolic blood pressure, mmHg*	143 \pm 10
Diastolic blood pressure, mmHg	86 \pm 11
Duration of diabetes mellitus (yr)*	7.2 \pm 2.5
BMI*	32 \pm 4
HbA1C*	7.0 \pm 0.9
Total Cholesterol (mg/dl)*	221 \pm 72
LDL-Cholesterol (mg/dl)*	146 \pm 14
HDL-Cholesterol (mg/dl)*	46 \pm 8
Triglyceride (mg/dl)*	163 \pm 53
Coexisting illness- no (%)	
Diabetes mellitus	43 (100)
Coronary heart disease	43 (100)
Peripheral artery disease	15 (35)
Hypertension	36 (84)
Smoking	4 (10)
Medication at enrollment-no (%)	
Insulin	10 (23)
Sulfonylurea	13 (30)
Metformin	33 (77)
Pioglitazone	8 (20)
Aspirin	38 (89)
ACE inhibitor †	23 (53)
ARB‡	14(33)
Beta-blocker	21 (49)
Calcium-channel blocker	10 (23)

*Values are given as mean \pm standard deviation,

†ACE, Angiotensin converting enzyme inhibitor

‡ARB, Angiotensin receptor blocker

immunometric assay on the Immulite 1000 Siemens analyzer (Siemens Medical Solutions Diagnostics Limited, Los Angeles, CA, USA). All blood samples were collected at 8:00 AM after a ten-hour fasting period using an intravenous line. The collected samples were centrifuged in a Heraeus Labofuge 400K (Thermo Fisher Scientific Inc. Waltham, MA, USA) at 4000g for 15 minutes and the sera were stored in a freezer at -80 °C (Thermo Fisher Scientific Inc. Waltham, MA, USA) until assay.

Statistical Analysis

Data were given as mean±standard deviation. The paired samples t-test was used to compare lipid levels and proinflammatory cytokines before and after treatment. The categorical data were compared using chi-square test and continuous data were analyzed with Student's t-test or Mann-Whitney U test, where applicable. Repeated measures ANOVA was used for different time points of treatment. When this test revealed a significant difference between the time points, pairwise comparisons were used to define which time points differ from which others. Bonferroni adjustment was applied for multiple testing. All statistical tests were two-sided and a p value lower than 0.05 was considered statistically significant. Statistical analyses were performed with SPSS® 11.5 for Windows™ (SPSS Inc., Chicago, IL).

Result

Influence of Atorvastatin on Serum Lipid Parameters

As expected, atorvastatin treatment led to a significant reduction in LDL-cholesterol levels at the end of the study: from 146±14 mg/dl at baseline to 67±5 mg/dl at week 12 (53.7% reduction, $p<0.001$). Plasma HDL-cholesterol levels unexpectedly decreased from 46±8 mg/dl to 41±7 mg/dl (11% reduction, $p<0.05$). Plasma triglyceride levels were reduced from 163±53 mg/dl to 107±36 mg/dl (34% reduction, $p<0.001$).

Effect of Atorvastatin on Pro-inflammatory Cytokines and hsCRP Levels

The treatment of patients with 80 mg of atorvastatin for 12 weeks ($n=43$) led to a marked decrease in the two peptide cytokines: for TNF (pg/ml), from 14.1±2.8 to 11.4±1.7 (22% reduction, $p=0.051$) and for IL-6 (pg/ml), from 4.2±1.9 to 3.6±1.7 (15% reduction, $p<0.05$).

Table 2. The effect of high dose atorvastatin treatment on the mean plasma level of TNF- α , IL-6, hsCRP, and lipid parameters*

Parameters	Before treatment	After treatment	P value
Total- Cholesterol (mg/dl)	221±72	127±8	$p<0.001$
LDL-Cholesterol (mg/dl)	146±14	67±5	$p<0.001$
HDL-Cholesterol (mg/dl)	46±8	41±7	$p<0.005$
Triglyceride (mg/dl)	163±53	107±36	$p<0.01$
TNF- α (pg/ml)	14.1±2.8	11.4±1.7	$P=0.051$
IL-6 (pg/ml)	4.2±1.9	3.6±1.7	$p<0.05$
hsCRP (mg/L)	2.8±1.1	1.2±0.3	$p<0.001$

*The values are given as mean±standard deviation

Atorvastatin treatment for 12 weeks resulted in a significant reduction in CRP levels at the end of the study: from 2.8±1.1 at baseline to 1.2±0.3 at week 12 (57% reduction, $p<0.001$).

Effect of Atorvastatin on Muscular and Hepatic Enzymes

No patients discontinued treatment due to perceived side effects, and no adverse or unexpected events occurred. The levels of CK, ALT, and AST remained within the normal ranges throughout the study.

Discussion

The leading cause of mortality in type 2 diabetic patients is atherosclerotic cardiovascular diseases (9-11). Dyslipidemia, characterized by increased plasma triglycerides, decreased HDL cholesterol, and increased small dense LDL particles is the major factor leading to cardiovascular events in type 2 diabetes (12-13). The mechanism by which tight glycemic control can play a role in preventing macrovascular complications is still questionable (14-15). Therefore, the importance of improving diabetic dyslipidemia should be emphasized in type 2 patients in order to prevent macrovascular complications. The LDL cholesterol is the major atherogenic lipoprotein and the National Cholesterol Education Program (NCEP) indicates LDL cholesterol as a target for lipid-lowering treatment. Very low level of LDL (<70mg/dl) is advised in patients with diabetes, especially if accompanied by coronary artery disease (6, 7). Statins are the drugs of choice for diabetic dyslipidemia due to their LDL-lowering and pleiotropic (restoring endothelial dysfunction, decreasing oxidative and inflammatory stress) effects. However, it is not clear whether statins' effects on inhibiting the pro-inflammatory response are maintained at very low levels of LDL. Previous studies have shown that statins decrease plasma pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 in addition to hsCRP (4, 16-19).

The present study shows that anti-inflammatory effects of high-dose atorvastatin (80 mg/day) treatment targeting very low LDL level are well maintained. After 12 weeks of atorvastatin treatment, the plasma hsCRP and IL-6 levels were significantly lowered. The decrease in TNF- α was, however, not statistically significant.

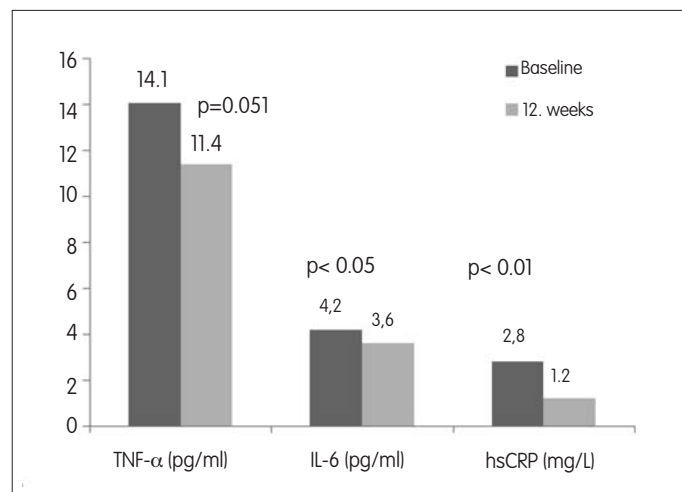


Figure 1. Effect of high dose atorvastatin therapy on plasma concentrations of TNF- α , IL-6, and hsCRP in patients with type 2 diabetes and coronary heart disease

There was a strong correlation between the percent reductions in LDL cholesterol and in CRP levels. The absolute reduction (1.6 mg/l) as well as the relative reduction (-57%) in the CRP levels with 80 mg of atorvastatin treatment detected in the present study is significantly greater compared with the previous studies (4, 16-19). The decrease in the plasma hsCRP levels was more significant compared to other studies and may be explained by two factors: First, most patients (89%) had taken aspirin, which anti-inflammatory effect might have increased this decline. Second, targeting very low levels of LDL might have helped decline in the plasma hsCRP levels. Considering that CRP facilitates uptake of oxidized LDL by macrophages, increases expression of vascular-cell adhesion molecules, and plays a role in progression of atherosclerosis by inhibiting nitric oxide synthesis independent from atherogenic lipoproteins, statins, in addition to LDL lowering effects, hold promise for the treatment of diabetic dyslipidemia with the aid of CRP decline even at very low LDL levels (20-23). Changes in pro-inflammatory cytokine IL-6 had weak but significant correlations with the reduction in LDL levels in regression analysis. Decline in IL-6 level was similar to the previous studies (absolute decline 0.6 pg/ml and relative decline 15%). The decline in plasma hsCRP did not correlate well with the decline in IL-6. This might be due to the role of some cytokines in the expression of hsCRP in the liver in addition to IL-6 (24). Further to the changes in other pro-inflammatory cytokines, TNF- α had weak but also nonsignificant correlations with the reduction in LDL levels. Considering that the effects of statins on pro-inflammatory markers start after 12 weeks of treatment, the nonsignificant p value might have been due to the limited number of patients despite a 22% relative decline in the TNF- α level. In conclusion, anti-inflammatory effect of high-dose statin treatment targeting very low LDL levels is maintained despite the fact that the number of patients and duration of treatment are among the limitation factors.

Disclosure: For all authors, there is no conflict of interest to disclose.

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