Atorvastatin improves metabolic control and endothelial function in Type 2 diabetic patients: A placebo-controlled study

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ABSTRACT. Several pieces of evidence support a role of inflammatory processes in the pathogenesis of atherosclerosis; it is also known that endothelial dysfunction is the initial lesion of the atherosclerotic process. Among other markers of endothelial dysfunction, some adhesion molecules seem to play an interesting role. The aim of the present study was to evaluate the effect of atorvastatin *vs* placebo on some indexes of leukocytes adhesion in a group of Type 2 diabetic patients. Twenty-five Type 2 diabetic patients free from microangiopathic complications and with LDL-cholesterol lower than 180 mg/dl were randomized to receive either atorvastatin ($T2D_A$) or placebo ($T2D_P$) for twelve months. BMI, fasting plasma glucose, glycated hemoglobin (HbA_{1c}), albumin excretion rate (AER), lipid profile, and serum concentrations of vascular cell adhesion molecule-1 (VCAM1), E-selectin and cadherin-5 were measured at baseline and at the end of the follow-up. At T_0 E-selectin was 16±6 ng/ml in $T2D_A$ and 17±13 in $T2D_P$; VCAM1 was 413±112 ng/ml in $T2D_A$ and 411±112 in $T2D_P$. At T_{12} VCAM1 and E-selectin did not vary in $T2D_P$, while a significant reduction was observed in $T2D_A$ (VCAM1 275±104 ng/ml and E-selectin 8±3 ng/ml; $p<0.001$ and $p<0.01$, respectively). $T2D_A$ also showed a reduction of total and LDL cholesterol and an improved glycemic control respect to $T2D_P$. Hypolipidemic therapy was the strongest independent predictor of the cytokines variations along the time. These results confirm the role of statins in modulating endothelial function also in Type 2 diabetes, outlining a therapeutic role of these molecules probably independent from the hypolipidemic effect.

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INTRODUCTION

Among the recent advances in understanding atherogenesis, the "inflammatory hypothesis" is one of the most interesting. Early stages in atherosclerosis, both in animal models and in human atherosclerotic tissues, are characterized by a specific interaction between endothelium and circulating leucocytes mediated by TNF-inducible adhesion molecules (1), such as E-selectin and circulating forms of CAMs. These molecules sustain the rolling, adhesion and migration of the leucocytes (2, 3) and require activation of the NF-kB family of transcription factors for complete expression.

The physiological meaning of these molecules is poorly understood but some evidence has shown a strong relationship with some pathological conditions (4), suggesting their potential role as markers of endothelial activation or even a molecular marker of early atherosclerosis (5).

Type 2 diabetes mellitus is associated with an increased risk of premature atherosclerosis; however, the mechanism through which diabetic patients develop vascular lesions is still unclear. Circulating levels of some adhesion molecules are higher in diabetic patients (6-7) suggesting an activated state of endothelium. A direct effect of hyperglycemia in influencing expression of some adhesion molecules has been described (8) but others factors could induce endothelial dysfunction in diabetes such as advanced glycosylation end products (9).

The beneficial effects of hydroxymethylglutaryl coenzyme A (HMGCoA) reductase inhibitors in reducing cardiovascular risk have been clearly established in several clinical trials for primary and sec-

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ondary prevention (10, 11). The mechanisms responsible for these clinical benefits, only partially known, may involve processes independent from lipid lowering effect. In particular, statins could modify endothelial functions, smooth muscle cells migration and proliferation, inflammatory response through direct anti-atherogenic effect in the arterial wall (12). This effect might be translated into a more significant prevention of cardiovascular disease in high-risk populations, including subjects with diabetes. Few studies, however have been performed in diabetic patients to clarify the effect of statin treatment on some functional activities peculiar of endothelium. To give a contribution to address this issue, we designed a study aimed at evaluating the short-term effects of atorvastatin in influencing endothelial function in a group of Type 2 diabetic patients.

SUBJECTS AND METHODS

25 patients (15 males and 10 females) with Type 2 diabetes were included in this study. Inclusion criteria were HbA_{1c} less than 9%, absence of micro (normal urinary albumin excretion rate, absence of retinopathy by fundoscopy) or macroangiopathic complications (no personal history of major cardiovascular or cerebrovascular events, normal resting ECG, carotid intima-media thickness lower than 0.9 mm in a B-mode ultrasonic scanning), and LDL-cholesterol<180 mg/dl. Patients assuming ACE-inhibitors were excluded from the study.

One patient was treated only with diet; 14 patients were treated with a combination of sulphonylurea plus metformin; 3 with metformin and 7 with sulphonylurea only. The therapeutic regimen for diabetes remained unaltered for the whole duration of the study, unless to register, at the end of the sixth month of treatment of HbA_{1c} over 1% respect to the basal value, that was considered a drop-out cause.

Table 1 - *Clinical characteristics of the two groups at baseline.*

	$T2D_A$	$T2D_P$
Age (yr)	66±8	63±9
Sex (M/F)	7/6	8/4
Diabetes duration (yr)	10±4	9±4
Hypertension (%) 54	54	50
Smokers (n°.)	4	2
Diabetes treatment (Diet/SU/Met/SU+Met)	0/4/2/7	1/3/1/7

$T2D_A$: atorvastatin 10 mg; TSD_P: placebo.

During the run-in period (3 weeks) the patients underwent a complete physical examination; moreover we recorded BMI and BP values.

After two weeks of a common treatment with diet and placebo, patients were randomized to receive atorvastatin 10 mg ($T2D_A$) or placebo ($T2D_P$) for 12 months. At the beginning (T_0) and at the end of the treatment (T_{12}) a venous blood sample was drawn for the determination of fasting plasma glucose (by glucose oxidase method), HbA_{1c} (by high performance liquid chromatography), lipid pattern (by standard enzymatic methods), renal profile. Albumin excretion rate (AER) was determined by RIA method in two consecutive 24-h urine collections at baseline and at the end of the protocol. Serum concentration of vascular cell adhesion molecule-1 (VCAM-1) E-selectin and cadherin-5 were measured using commercial immunoassays by ELISA (MedSystem Diagnostic, Wien, Austria). The intra-assay coefficients for these determinations were less than 5%.

We also estimated glucose disposal rate (GDR) in all patients at baseline and at the end of the treatment as indicated by Williams

Table 2 - *Clinical and biochemical parameters of the two groups at baseline and at the end of the follow-up.*

	$T2D_A$		$T2D_P$	
	T_0	T_{12}	T_0	$T1_2$
BMI (kg/m^2)	30.0±3.2	29.5±3.2	28.4±2.1	28.4±2.5
Systolic blood pressure (mmHg)	145±17	138±18	135±12	133±20
Diastolic blood pressure (mmHg)	83±12	78±5	85±7	82±9
Fasting plasma glucose (mg/dl)	200±45	189±34	178±53	183±54
HbA_{1c} (%)	8.6±1.2	7.7±1.0*	8.2±1.3	7.8±1.3
Total cholesterol (mg/dl)	236±53	190±48*	203±27	208±39
HDL-cholesterol (mg/dl)	55±11	56±15	49±12	48±13
LDL-cholesterol (mg/dl)	149±51	108±40*	122±22	129±33
Plasma triglycerides (mg/dl)	162±70	130±64*	137±60	156±103
BUN (mg/dl)	34±7	35±8	40±12	43±8
Plasma creatinine (mg/dl)	1.02±0.2	1.03±0.2	1.11±0.2	1.01±0.1
AER (µg/min)	4 (1-16)	6 (2-15)	5 (2-13)	11(6-21)
Estimated GDR (mg/kg/min)	5.46±2.28	6.27±1.74*	6.52±2.20	6.45±2.26

AER: albumin excretion rate; BUN: blood urea nitrogen.

et al. (13). Based on this model, the GDR (expressed in mg·kg^{-1}·min^{-1}) can be calculated as follows:
24.31 – 12.22 (WHR) – 3.29 (HTN) – 0.57 (HbA_{1c})
where WHR is waist-to-hip ratio, HTN is personal history of hypertension (0=no, 1=yes), and HbA_{1c} level is expressed as %.
The study protocol was approved by the Ethical Committee of the University of Ferrara School of Medicine. All participants in the study gave their written informed consent.

STATISTICAL ANALYSIS

Data are expressed as mean±SD. Differences between groups were evaluated using one-way ANOVA for repeated measures. Variations of clinical parameters within the same groups during follow up were tested by *t* test for paired data. Relationship between cytokines and other variables of interest was evaluated by Pearson and Spearman rank correlation tests. A multiple regression analysis was employed to evaluate the independent effect of several variables on cytokines variation. A *p* value less than 0.05 was considered statistically significant. Analyses were performed using SPSS 10.0 for Windows.

RESULTS

All enrolled patients completed the study. No side-effects were reported; particularly neither an increase of creatinphosphokinase nor alteration of hepatic function were described. We did not register any significant increment of HbA_{1c} during the follow-up in any patients; consequently they did not vary their antihyperglycemic therapy along the whole duration of the study.
Clinical characteristics of the two study groups are shown in Table 1. They were well matched for age, sex, presence/absence of hypertension and duration of disease.
Table 2 shows biochemical parameters at baseline and at the end of the follow up. At T_0, $T2D_A$ and $T2D_P$ had the same degree of metabolic control, as shown by fasting plasma glucose and HbA_{1c}; similarly, there were no differences in lipid pattern. At T_{12}, as expected, $T2D_A$ showed significantly lower total and LDL-cholesterol levels and lower triglycerides respect to T_0 values (all $p<0.001$). Moreover, despite no variation in therapy, $T2D_A$ patients showed an improved metabolic control, as shown by a decreased value of HbA_{1c} ($p<0.001$ with respect to T_0); at the meantime in these patients a significant increase of GDR was also registered (Table 2, $p<0.001$). No patient, either in placebo or in atorvastatin group, developed microalbuminuria during one year of observation.
In Figure 1 the serum concentrations of VCAM1 (panel A), E-selectin (panel B) and cadherin-5 (panel C) at T_0 and T_{12} are reported. At baseline there were no differences in serum adhesion molecules concentrations between groups. Respect to T_0, at the end of the study, neither variations of E-selectin nor VCAM1 or cadherin-5 levels were observed within $T2D_P$ group, while $T2D_A$ patients showed a significant decrease of VCAM-1 and E-selectin (both $p<0.0001$).

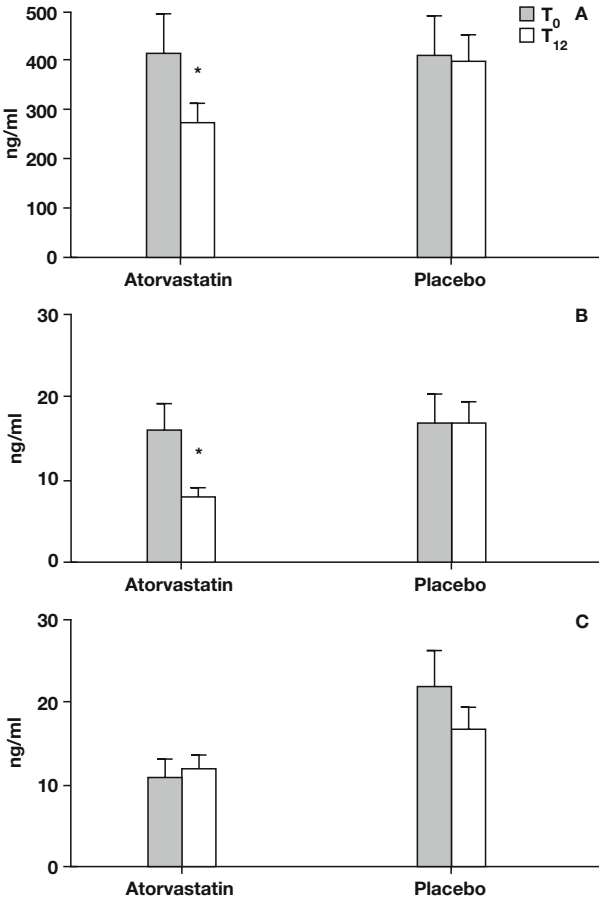

Fig. 1 - *Serum concentrations of vascular cell adhesion molecule-1 (VCAM-1) (panel A), E-selectin (Panel B) and cadherin-5 (panel C) at the baseline (grey bars) and at the end of the follow-up (white bars) in the two groups of patients.*

At baseline we did not observe any significant correlation between adhesion molecules levels and any clinical or biochemical parameter. At T_{12}, E-selectin and cadherin-5 were significantly related to plasma triglycerides (r=0.423 and r=0.645; $p<0.05$ and $p<0.001$, respectively).
In order to evaluate the role of the different metabolic parameters in influencing cytokine levels, we used three stepwise models where we inserted as dependent variables respectively △VCAM1 ($VCAM1_{T12}$-$VCAM1_{T0}$), △E-selectin ($E\text{-}selectin_{T12}$-$E\text{-}selectin_{T0}$), and △cadherin-5 ($cadherin\text{-}5_{T12}$-$cadherin\text{-}5_{T0}$). Data are re-

Table 3 - *Regression analysis showing the relationship between deltas of cytokine concentrations along the follow-up (T_{12}-T_0) and other variables.*

	△VCAM1 (R^2=0.665)		△E-Selectin (R^2=0.688)		△Cadherin (R^2=0.426)	
	Standardized β coefficient	*p* value	Standardized β coefficient	*p* value	Standardized β coefficient	*p* value
Lipid lowering therapy	-0.695	<0.001	-0.708	<0.001	-0.393	0.06
Age	0.255	0.24	-0.474	<0.05	-0.260	0.36
Hypertension (yes/no)	-0.012	0.95	0.424	<0.05	-0.234	0.39

Independent variables included in the three models: age, △BMI, △HDL, △triglycerides, $\triangle HbA_{1c}$, presence/absence of hypertension, lipid lowering treatment; VCAM-1: vascular cell adhesion molecule-1.

ported in Table 3. Lipid lowering therapy was the only parameter behaving as independent predictor on variation of the three cytokines along the time (for VCAM1 and E-selectin both $p<0.001$ and $p=0.06$ for cadherin-5); moreover, △E-selectin was predicted by age and presence of hypertension.

DISCUSSION

This study shows that in Type 2 diabetic patients a 12 month treatment with atorvastatin is able to reduce serum concentrations of E-selectin and VCAM-1; this effect is not patent in a group of patients treated with placebo. No differences were observed in cadherin-5 concentrations either in patients treated with placebo or drug.

E-selectin is found only on activated endothelium (14); its demonstration in the blood would be taken as conclusive evidence of endothelial activation. In previous cross-sectional studies E-selectin levels have been described significantly higher in patients with Type 2 diabetes than in normal subjects and reduced by a sustained improvement in glycemic control (15). The decrease was independent of the pharmacological agents (glibenclamide, metformin, insulin). We were unable to confirm these observations: in our patients, levels of E-selectin were comparable to those obtained in the same laboratory in a group of 75 normal subjects (21±12 ng/ml).

VCAM-1 is a transmembrane glycoprotein member of the immunoglobulin superfamily; its increased concentrations could reflect processes mainly located at the level of the intima of the arterial wall. Previous studies have demonstrated increased plasma levels of some CAMs in diabetic patients, although a definite pattern has not emerged (16); on the other hand, Sardo *et al.* (17), using simvastatin, have recently failed to demonstrate a reduction of VCAM-1. In that study, actually, patients were treated for a shorter period of time, outlining the importance of a prolonged therapy to powerfully influence endothelial function in these patients.

Concerning the mechanisms inducing the reduction of the two cytokines, it might be either due to a direct effect of atorvastatin on vascular wall or being an indirect effect of improved metabolic control observed in these patients at the end of the follow up. Recently, Ryssy *et al.* (18) have shown that in Type 2 diabetic patients an amelioration in glucose control is able *per se* to reduce serum E-selectin but not VCAM levels. Even though we lacked to find any direct correlation between △E-selectin and $\triangle HbA_{1c}$, probably due to the small number of patients, a reduction in HbA_{1c} levels of $T2D_A$ was observed, in absence of any variation of diabetes regimen during the follow-up in the two study groups, making conceivable that different mechanisms might underlie the reduction of these two compounds.

Cadherin-5 is a cell surface protein directly involved in a wide variety of processes such as cell adhesion, cell survival, formation of intercellular junctions, maintenance of tissue integrity, angiogenesis (19). Our study failed to show any difference in cadherin-5 levels in the two study groups, irrespective of the treatment. The meaning of circulating levels of this protein are however still dubious.

Other two results of our study, in our opinion, deserve attention. The first one is a reduced insulin resistance in patients treated with atorvastatin. Previous reports have described an inverse relationship between insulin sensitivity and E-selectin in Type 2 diabetes (20); unfortunately, we could not perform a direct measurement of insulin sensitivity in our patients, *i.e.* by a glucose clamp, and we are aware the limit of the Williams index, deeply influenced by fasting glucose concentrations. Our data, however, do not allow to certainly ascribe the effect on insulin sensitivity to a direct action of atorvastatin, rather than to the improved metabolic control. It is also conceivable, however, to hypothesize other possible mechanisms: for example, the triglyceride-lowering effect of atorvastatin therapy may be important *per se* in reducing development

and/or progression of insulin resistance along the time, but – more probably – the anti-inflammatory effect of the compound might be crucial. Statins have been shown to reduce circulating levels of inteleukin-6 and TNFα (21), chemokines known to inhibit lipoproteinlipase activity and to stimulate lipolysis in adipose tissue (22). The anti-inflammatory properties of atorvastatin may therefore interrupt the natural progression from central obesity to insulin resistance mediated by the adipose tissue derived cytokines. TNFα is also able to induce expression of E-selectin by endothelial cells (23); consequently atorvastatin could have induced the reduction of E-selectin also via this pathway.

Another possibility could be the direct effect of HMGCoA reductase inhibitors on endothelium, given that an impaired endothelial function has recently been shown to result in diminished capillary recruitment and in turn to correlate with the degree of insulin resistance (24). By improving endothelial function, atorvastatin may significantly influence selective tissue perfusion and thereby beneficially affect glucose and insulin transport, also contributing to improve metabolic control in these patients.

The other observation is a linear relationship between plasma triglycerides and both E-selectin and cadherin-5 levels at the end of the treatment period in all the study population, either attributable to the strict link between metabolic control and plasma triglycerides in Type 2 diabetic patients and to the effect of atorvastatin on reduction of apolipoprotein B-containing lipoproteins secretion, mechanism probably responsible for the triglyceride-lowering effect of the compound (25).

A multiple regression analysis has indicated the presence of lipid-lowering therapy as the main determinant of the variation of cytokines levels along the time; an effect of age and presence of hypertension on E-selectin variations was also patent. Several Authors, mainly in cross-sectional studies, have described higher E-selectin levels in patients with essential hypertension than in normotensive subjects (26, 27); our observations suggest that in Type 2 diabetic patients, hypertension as part of an insulin resistance syndrome, might exert a more profound impact on cytokine variations, even over time.

In conclusion, this double-blind placebo-controlled study confirms an anti-inflammatory effect of atorvastatin also in Type 2 diabetes, probably via an improvement of endothelial function. Further analysis is necessary to examine whether this reduction of cytokines circulating levels plays biological roles in some physiological or pathological conditions.

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