SCIENTIFIC LETTER

Effects of atorvastatin and vitamin C on forearm hyperaemic blood flow, asymmentrical dimethylarginine levels and the inflammatory process in patients with type 2 diabetes mellitus

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Heart 2007;**93**:244–246. doi: 10.1136/hrt.2006.093112

Type 2 diabetes mellitus (DM) is characterised by increased oxidative stress as a result of the hyperglycaemic state, leading to decreased nitric oxide bioavailability.[1] Similarly, type 2 DM is characterised by increased levels of proatherogenic cytokines and adhesion molecules,[1] while it has been shown that the endogenous endothelial nitric oxide synthase inhibitor, asymmetrical dimethyl arginine (ADMA), is also elevated in type 2 DM.[2] Although ADMA synthesis is triggered by both oxidative stress and proinflammatory stimuli, the effect of antioxidant or anti-inflammatory treatment on its release is unclear.

We compared the effects of atorvastatin (which has anti-inflammatory properties in atherosclerosis) and vitamin C (a well-known antioxidant) on the inflammatory process, endothelial function and the release of ADMA in normocholesterolaemic patients with type 2 DM.

METHODS

Forty-one patients with type 2 DM and no evidence of macroangiopathy were recruited (table 1). The absence of coronary artery disease (CAD) was established by a negative exercise stress test within the last 6 months before recruitment, while peripheral artery disease was defined as an ankle brachial index <0.90. All patients had cholesterol <5.4 mmol/l at baseline, and exclusion criteria were the use of statins, insulin, antioxidant supplements, hormone replacement therapy or anti-inflammatory medication during the past year, or the presence of any chronic disease or infection. All subjects had normal renal function (normal creatinine clearance as calculated by the Cockroft-Gault formula and no macroalbuminuria). Patients were randomly allocated into groups receiving atorvastatin (10 mg/day), vitamin C (2 g/day) or no treatment (controls) for 4 weeks, in a single-blinded design (the researchers were blinded to the patients' treatment group). All studies were conducted between 08:00 and 10:00 h after a 12-h fasting period, and the last medication was administered the night before the follow-up visit. Forearm blood flow (FBF), forearm vasodilatory response to post-ischaemic hyperaemia (per cent change in reactive hyperaemia, RH%) and endothelium independent dilation (per cent change in nitroglycerin-mediated dilation, NTG%) were assessed by gauge strain plethysmography as we have previously described.[3] The inter- and intra-observer variability for this method in our laboratory is <3%. Routine chemical methods were used to determine serum concentrations of total cholesterol, high density lipoprotein (HDL), triglycerides and glucose (colourimetric enzymatic method; Technicon automatic analyser RA-1000, Date-Behring Marburg, Marburg, Germany). ELISA was used to determine serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) (ELISA kits by R&D Systems, Minneapolis, Minnesota, USA) and ADMA (DLD Diagnostika, Hamburg, Germany). The specificities of all the used ELISA kits were ~100% and the sensitivities for the ADMA, TNF-α, IL-6 and sVCAM-1 kits were 0.05 μmol/l, 0.06 pg/ml, 0.0094 pg/ml and 0.17 ng/ml, respectively.

The protocol was approved by Institutional Ethics Committee, and informed consent was given by each subject. All statistical analyses were performed using the SPSS statistical package for Windows, version 12.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Atorvastatin treatment decreased serum IL-6, TNF-α, sVCAM-1, C-reactive protein (CRP) and ADMA while no change was observed in the vitamin C or control groups (table 1). The changes in cholesterol levels after treatment were correlated with the alterations in TNF-α ($r = 0.359$, $p = 0.023$), IL-6 ($r = 0.401$, $p = 0.001$) and sVCAM-1 ($r = 0.335$, $p = 0.034$), while no correlation between the change in cholesterol and ADMA levels was observed ($r = 0.242$, $p = 0.124$). Furthermore, a significant correlation was found between the decrease in TNF-α and the decrease in ADMA levels ($r = 0.416$, $p = 0.008$).

Atorvastatin increased maximum hyperaemic FBF and RH%, but it had no effect on resting FBF or NTG% (table 1). Vitamin C had a borderline but not significant effect on RH% ($p = 0.08$), while it had no effect on resting or hyperaemic FBF (table 1). The changes in maximum hyperaemic FBF and RH% were not correlated with the changes in lipid levels. Similarly, no change was observed in RH% and resting or maximum hyperaemic FBF in the control group.

DISCUSSION

Type 2 DM is associated with endothelial dysfunction as a result of both decreased production and increased oxidative inactivation of nitric oxide by free radicals.[1] A new pathophysiological pathway contributing to endothelial dysfunction in type 2 DM is mediated by the increase in ADMA.[2] It is believed that oxidative deactivation of dimethylarginine dimethylaminohydrolase, the enzyme responsible for ADMA metabolism, is a key mechanism by which type 2 DM induces the release of ADMA.[2] Furthermore, ADMA is partly cleared by renal excretion, and

Abbreviations: ADMA, asymmetrical dimethyl arginine; CAD, coronary artery disease; CRP, C-reactive protein; DM, diabetes mellitus; FBF, forearm blood flow; HDL, high density lipoprotein; IL-6, interleukin-6; NTG%, per cent change of flow after nitrate administration; RH%, per cent change of flow during reactive hyperaemia; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF-α, tumour necrosis factor-α

Table 1 Demographic characteristics of the participants and effects of treatment on endothelial function, ADMA levels and the inflammatory process

	Atorvastatin-treated (n = 15)		Vitamin C-treated (n = 13)		Controls (n = 13)	
Demographic characteristics						
Age (years)	58.1 (2.6)		59.1 (2.4)		60.9 (3.1)	
Sex (males/females)	8/7		6/7		8/5	
Duration of diabetes (years)	8.3 (0.8)		8.6 (0.9)		7.7 (0.9)	
BMI (kg/m^2)	27.5 (0.55)		29.1 (1.1)		28.0 (0.58)	
HbA1c (%)	6.80 (0.29)		6.29 (0.22)		6.67 (0.23)	
Creatinine clearance (ml/min)	94.2 (4.7)		101.5 (11.2)		97.1 (8.7)	
Current smokers	6		7		7	
Hypertension	6		6		7	
	Before	**After**	**Before**	**After**	**Before**	**After**
Effects of treatment						
Resting FBF1 (ml/100 ml tissue/min)	4.07 (0.49)	4.33 (0.507)	4.94 (0.44)	5.28 (0.47)	4.59 (0.37)	4.71 (0.28)
Maximum hyperaemic FBF (ml/100 ml tissue/min)	5.81 (0.71)	6.82 (0.80)*	7.59 (0.83)	8.33 (0.74)	6.57 (0.52)	6.93 (0.55)
RH% (%)	42.9 (4.9)	58.5 (4.8)**	51.3 (6.02)	59.0 (7.92)	43.9 (5.1)	46.1 (6.1)
Resting FBF2 (ml/100 ml tissue/min)	4.15 (0.37)	4.21 (0.39)	4.51 (0.35)	4.67 (0.36)	4.95 (0.25)	5.4 (0.46)
Maximum FBF after nitrate (ml/100 ml tissue/min)	7.62 (0.68)	7.71 (0.68)	7.53 (0.71)	7.61 (0.77)	8.87 (0.72)	9.48 (0.84)
NTG%	85.5 (0.39)	87.9 (0.08)	76.9 (6)	73.2 (0.80)	79.0 (12.6)	82.7 (14.8)
ADMA (μmol/l)	1.21 (0.18)	0.72 (0.08)*	1.02 (0.09)	0.98 (0.07)	0.95 (0.07)	0.93 (0.08)
sVCAM-1 (ng/ml)	484.7 (31.2)	363.5 (13.2)*	380.8 (61.2)	494.7 (32.3)	450.5 (37.2)	448.9 (48.9)
IL-6 (pg/ml)	2.27 (0.55)	1.36 (0.19)*	2.65 (0.42)	3.45 (0.44)	2.28 (0.59)	2.41 (0.51)
TNF-α (pg/ml)	2.51 (0.54)	1.62 (0.18)*	2.71 (0.87)	2.59 (0.49)	1.96 (0.22)	2.05 (0.19)
CRP (mg/l)	3.84 (1.16)	2.34 (0.71)*	2.39 (0.26)	2.32 (0.280	2.42 (0.69)	2.21 (0.73)
Cholesterol (mmol/l)	5.05 (0.22)	4.26 (0.27)*	4.88 (0.21)	5.12 (0.24)	4.54 (0.21)	4.33 (0.21)
Triglycerides (mmol/l)†	1.51 (0.93–1.75)	1.31 (0.87–1.57)*	1.69 (1.07–2.37)	1.60 (0.94–2.46)	1.46 (1.32–1.72)	1.41 (1.13–2.27)
LDL (mmol/l)	2.94 (0.23)	2.32 (0.32)	2.64 (0.43)	3.34 (0.25)	2.70 (0.28)	2.35 (0.24)
HDL (mmol/l)	1.42 (0.25)	1.45 (0.22)	1.36 (0.35)	1.14 (0.11)	1.38 (0.26)	1.34 (0.15)
Fasting glucose (mg/ml)†	6.8 (6.6–8.0)	7.4 (6.1–8.0)	7.0 (6.2–8.7)	7.3 (5.8–8.7)	7.0 (6.2–8.7)	7.3 (5.8–8.7)

ADMA, asymmetrical dimethylarginine; CRP, C-reactive protein; FBF, forearm blood flow; HDL, high density lipoprotein; IL-6, interleukin-6; NTG%, per cent change in flow after sublingual nitrate administration; RH%, forearm vasodilatory response to reactive hyperaemia; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF-α, tumour necrosis factor-α.
Values are expressed as means (SEM); †values expressed as median (25th–75th percentile).
*$p<0.05$, **$p<0.01$ *v* baseline.
There were no significant differences in baseline and demographic characteristics between the three groups after adjustment by using Bonferroni post-hoc analysis for three comparisons.

reduced clearance of ADMA due to diabetic nephropathy may be an additional mechanism of ADMA increase in type 2 DM.[4]

It has been proposed recently that statins may improve endothelial function through the decrease in ADMA levels in patients with hypercholesterolaemia.[4] The effect of statins on ADMA levels may be mediated by the decrease in oxidised LDL levels, while it has been suggested that the ability of statins to depress cytokine expression (and especially TNF-α) indirectly decreases ADMA levels. Furthermore, it was also shown that LDL stimulates the synthesis of ADMA in endothelial cells,[4] suggesting a direct relationship between lipids and ADMA production. However, the effect of statins on endothelial function and ADMA synthesis in normocholesterolaemic patients with uncomplicated type 2 DM is unknown.

In the present study a significant reduction in ADMA levels accompanied by an improvement in endothelial function was found in the atorvastatin group, an effect unrelated to lipid lowering. On the contrary, the decrease in ADMA levels was correlated with the respective decrease in TNF-α, suggesting that proinflammatory stimuli and especially TNF-α may be a connective link between statin treatment and the decrease in ADMA levels. We have also shown that classic antioxidant treatment is unable to modify ADMA levels and endothelial function, at least in this particular population.

Type 2 DM is associated with increased levels of proinflammatory cytokines (IL-6 and TNF-α), adhesion molecules (for example, sVCAM-1) and CRP.[1] In hypercholesterolaemic patients with type 2 DM, statins have antiinflammatory effects, but their role in normocholesterolaemic patients with type 2 DM is still unclear.[5] Recent data suggest that low-dose atorvastatin treatment (10 mg/day) is sufficient to reduce cardiovascular risk in patients with type 2 DM and low cholesterol levels, but this finding is not widely accepted.[5]

We have shown that 4-week treatment with atorvastatin significantly decreased the expression of IL-6, TNF-α, sVCAM-1 and CRP in patients with type 2 DM and normal cholesterol levels. This effect could be partly mediated by the reduction in lipid levels since the observed changes were significantly correlated with the respective changes in cholesterol levels, although the pleiotropic effects of atorvastatin cannot be excluded.

The study limitations include the absence of any measurements of oxidative stress markers, the open-labelled non-placebo controlled design of the study, and the relatively small number of participants in each treatment group. Furthermore, the presence of CAD was excluded by an exercise stress test, and some of the participants may have had sub-clinical ischaemic heart disease not detectable by this method.

In conclusion, atorvastatin (but not vitamin C) improved endothelial function and decreased the expression of IL-6, TNF-α, CRP, sVCAM-1 and ADMA in patients with type 2 DM, while the decrease in ADMA was correlated with the decrease in TNF-α. These findings imply that atorvastatin treatment may be beneficial in low-risk normocholesterolaemic patients with type 2 DM by improving endothelial function, decreasing ADMA levels and depressing the inflammatory process.

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Competing interests: None declared.

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Accepted 21 July 2006
Published Online First 16 August 2006

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IMAGES IN CARDIOLOGY

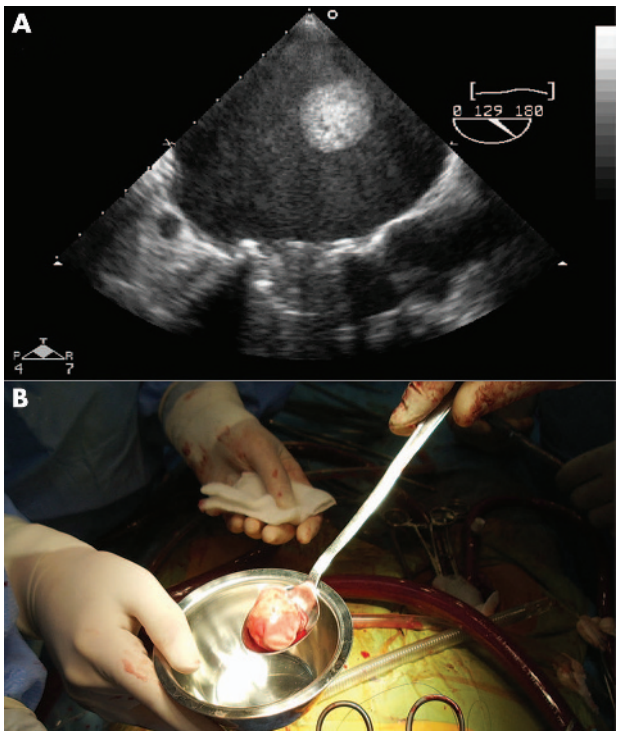
doi: 10.1136/hrt.2006.091306

Free-floating left atrial thrombus formed in a few weeks in a patient with a normal mitral prosthesis

A 53-year-old woman with rheumatic heart disease (having had a Bjork-Shiley 29 mitral prosthesis and a Braille 31 tricuspid bioprosthesis implanted in 1980 and 1995, respectively), in permanent atrial fibrillation, was electively admitted to our department for redo cardiac surgery. Ten weeks previously she was diagnosed with severe tricuspid stenosis (bioprosthesis degeneration), in New York Heart Association functional class III heart failure and was scheduled for cardiac reintervention. At that time mitral prosthesis function was normal and the left atrium (LA) was free of thrombi at transoesophageal echocardiography (TOE) (video 1; to view video footage visit the *Heart* website—http://www.heartjnl.com/supplemental).

Three weeks later the patient suffered a gastrointestinal haemorrhage. Anticoagulation was stopped, blood products were administered, her condition stabilised and anticoagulation was resumed, but the international normalised ratio (INR) was not checked thereafter. At current admission she was clinically stable and no embolic events or clinical hints suggestive of LA ball thrombus could be identified. The INR was 1.05. A free-floating LA thrombus was diagnosed by transthoracic echocardiography (video 2). Mitral prosthesis was normal and the tricuspid bioprosthesis had deteriorated and was severely stenotic. TOE showed the thrombus entering the mitral prosthesis intermittently, being hit by the disc and bouncing back into the LA (panel A, video 3).

At surgery, a 3 × 3× 4 cm free-floating thrombus (panel B) was removed from the LA and the tricuspid prosthesis was replaced. Recovery was uneventful. This is a very rare case of a large, clinically silent free-floating LA thrombus formed over a short period of time in a patient with a normal mechanical mitral prosthesis, stressing the importance of appropriate anticoagulation.



(A) Transoesophageal echocardiography showing the free-floating thrombus in the left atrium (see video 3). (B) The thrombus after its surgical removal from the left atrium.

To view video footage visit the *Heart* website—http://www.heartjnl.com/supplemental

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