

Effects of atorvastatin and vitamin C on endothelial function of hypercholesterolemic patients

Francesco Perticone *, Roberto Ceravolo, Raffaele Maio, Cosima Cloro, Mafalda Candigliota, Angela Scozzafava, Annalisa Mongiardo, Pasquale Mastroberto ^a, Massimo Chello ^a, Pier L. Mattioli¹

Cardiovascular Diseases Unit, Department of Medicina Sperimentale e Clinica 'G Salvatore', Policlinico Mater Domini, Via Tommaso Campanella, University of Catanzaro, 88100 Catanzaro, Italy

^a Cardiac Surgery Unit, Department of Medicina Sperimentale e Clinica 'G Salvatore', Policlinico Mater Domini, Via Tommaso Campanella, University of Catanzaro, 88100 Catanzaro, Italy

Received 2 August 1999; received in revised form 12 November 1999; accepted 16 December 1999

Abstract

We tested the effects of vitamin C and atorvastatin treatment on endothelium-dependent and endothelium-independent vasodilation in 18 hypercholesterolemic patients (ten men and eight women, aged 20–46 years) in comparison with 12 normal volunteers (seven men and five women, aged 20–45 years). The responses of the forearm blood flow (FBF) to acetylcholine (ACh) (7.5, 15 and 30 $\mu\text{g}/\text{min}$), sodium nitroprusside (SNP) (0.8, 1.6, 3.2 $\mu\text{g}/\text{min}$) and L-NMMA (2, 4, 8 $\mu\text{mol}/\text{min}$) were evaluated at baseline and after 1 month of atorvastatin (10 mg/day) treatment. Drugs were infused into the brachial artery and FBF was measured by strain-gauge plethysmography. At baseline, the response to ACh was significantly attenuated in hypercholesterolemics versus controls: at the highest dose (30 $\mu\text{g}/\text{min}$), FBF was 27.0 ± 3.4 versus 11.5 ± 1.9 ml·100 ml tissue⁻¹·min⁻¹ respectively ($P < 0.0001$). No significant differences were found between groups during SNP infusion. The atorvastatin treatment significantly improved ACh-stimulated FBF: at highest dose the FBF increased to 14.9 ± 1.5 ml·100 ml tissue⁻¹·min⁻¹ ($P < 0.0001$). Similarly, the L-NMMA endothelial effects were significantly enhanced by lipid-lowering treatment, supporting the improvement of basal nitric oxide. Vitamin C increased ACh-vasodilation in the same way before and after atorvastatin treatment. In conclusion, the endothelial dysfunction in hypercholesterolemics is due to an oxidative stress and atorvastatin rapidly improves both basal and stimulated endothelium-dependent vasodilation. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hypercholesterolemia; Endothelium; Atorvastatin; Atherosclerosis; Vitamin C

1. Introduction

The normal endothelium plays a key role in the local regulation of the vascular tone by producing and releasing contracting and relaxing agents [1]. One of these factors is the nitric oxide (NO) [2–4] that is released after stimulation of endothelial cells by shear stress [5], as well as some agonists such as acetylcholine (ACh), bradykinin, substance P and serotonin [4]. On the other

hand, sodium nitroprusside (SNP) is an endothelium-independent vasodilator compound that produces vasodilation by direct activation of guanylate cyclase in vascular smooth-muscle cells by providing inorganic source of NO [6].

Endothelium-dependent vasodilation has been shown to occur in most mammalian species [2] and in humans, by in vitro studies using arterial preparations [7,8]. Human studies demonstrated that this regulatory action of the endothelium is exerted on resistance vessels too [9]. Moreover, this endothelial function is impaired in the presence of different risk factors such as hypertension [10–12], cigarette smoking [13], diabetes [14] and hypercholesterolemia [12,15]. A dysfunctioning endothelium reduces its ability to exert a protective effect

¹Professor Mattioli suddenly died on June 23, but he actively participated in the present study.

* Corresponding author. Tel.: +39-0961-712264; fax: +39-0961-775564.

E-mail address: perticone@unicz.it (F. Perticone).

on the vascular system by keeping vessels in a dilatory state and playing, thus, a key pathophysiological role in the development of atherosclerosis. Recently, improvement of endothelial dysfunction was demonstrated after various treatments such as antioxidants [16,17], estrogen replacement [18], lipid lowering drugs [17,19,20], angiotensin-converting enzyme inhibitors [21] and calcium-channel blockers [22,23].

Inhibitors of HMG-CoA reductase are currently extensively used to lower serum cholesterol levels and to improve long-term morbidity and mortality related to coronary artery disease (CAD) [24,25]. Atorvastatin, a new HMG-CoA reductase inhibitor, demonstrated great effectiveness in reducing total and LDL-cholesterol and triglyceride levels and in raising HDL-cholesterol [26]. Recently, Simons demonstrated, by reactive hyperemia, that atorvastatin improves forearm blood flow (FBF) in hypercholesterolemics [27]. However, at present no data are available about the atorvastatin effects on ACh-stimulated vasodilation by strain-gauge plethysmography.

Based on these observations, the aims of this study were to test in hypercholesterolemics without overt vascular disease: (1) the effect of vitamin C by intra-arterial infusion; (2) the effects on both basal endothelium-dependent vasodilation and ACh-induced vasodilation in the forearm vasculature after atorvastatin treatment; and (3) the combined effect of vitamin C and atorvastatin administration.

2. Methods

2.1. Study population

2.1.1. Hypercholesterolemic patients

We enrolled 18 hypercholesterolemic outpatients, ten men and eight women, aged 20–46 years (mean \pm S.D., 40 ± 6 years). Patients were eligible if they had total serum cholesterol levels > 6.2 mmol/l and were not receiving cholesterol-lowering medications. Patients were excluded if any of the following were present: diabetes mellitus, primary or secondary hypertension, CAD, heart failure, peripheral vascular disease, cigarette smoking, vasoactive drugs, antioxidant compounds, post-menopausal status or hormone replacement therapy.

2.1.2. Control subjects

Twelve normal volunteers, seven men and five women, aged 20–45 years (mean \pm S.D., 39 ± 5 years), were selected as a control group. Each patient underwent clinical history, physical examination, standard electrocardiogram, routine chemical analyses and chest X-rays. None had evidence of present or past hypertension, cardiovascular disease, hyperlipidemia, or any

other systemic condition and none was taking drugs at the time of the study.

2.2. Study protocol

The protocol was designed as a randomized, double blind, placebo-controlled crossover study. At first, FBF dose–response curves were evaluated in both groups (normal subjects and hypercholesterolemic patients) for ACh and SNP infusions before and during co-administration of vitamin C.

After the initial evaluation of vascular function, the hypercholesterolemic patients were randomized in equal numbers ($n = 9$) to receive for 4 weeks the same diet plus atorvastatin (10 mg daily) or placebo. At the end of this period, the vascular function was reevaluated in each patient. Then, after crossover, they were again studied, 4 weeks later. Biochemical and hematological parameters were obtained with the patients fasting at randomization and at the end of each treatment period.

To assess the reproducibility of endothelium-dependent and endothelium-independent vasodilation, vascular function in the control group was reevaluated 2 months later.

The local ethical committee approved the study, and all participants gave written informed consent for all procedures.

2.3. Vascular function

All studies were performed at 9:00 a.m. after overnight fasting, with the subjects lying supine in a quiet air-conditioned room (22–24°C), using the same protocol previously described by Panza [10] and successfully employed by ourselves [23,28].

All hypercholesterolemics and controls underwent measurement of FBF and blood pressure (BP) during intra-arterial infusion of saline and increasing doses of ACh and SNP. All participants rested at least 30 min after artery cannulation to obtain a stable baseline before data collection; FBF and vascular resistance (VR) were repeated every 5 min until stable.

Endothelium-dependent vasodilation was evaluated by dose–response curve to intra-arterial ACh infusions (7.5, 15 and 30 μ g/min, each for 5 min). Endothelium-independent vasodilation was assessed by dose–response-curve to intra-arterial SNP infusions (0.8, 1.6, 3.2 μ g/min, each for 5 min).

Vitamin C, at a constant dose of 24 mg/min, was infused via the brachial artery to evaluate its effect on vasodilatory responses to ACh and SNP. This vitamin C concentration has been shown to protect human plasma from free radical-mediated lipid peroxidation [16] and to improve the impaired ACh-induced vasodilation in smokers [29], in hypercholesterolemics [16,17], in hypertensives [30] and in diabetic patients [31].

Finally, vitamin C administration was followed, in hypercholesterolemic patients, by L-NMMA infusions (2, 4 and 8 $\mu\text{mol}/\text{min}$, each for 5 min) to assess the basal NO-mediated endothelium-dependent vasodilation before and after atorvastatin treatment.

The FBF and VR of the opposite arm were also evaluated during the study.

Infusion rate for each drug was 1 ml/min.

2.4. Drugs

ACh (Sigma, Milan, Italy), vitamin C (Bracco, Milan, Italy) and L-NMMA (Clinalfa, San Diego, CA) were obtained from commercially available sources and diluted in saline immediately before each infusion. SNP (Malesci, Florence, Italy) was diluted in 5% glucose solution and protected from light with aluminum foil.

2.5. Statistical analysis

The goal of the present study was the increase in FBF from the baseline values. Based on a single measurement in each step, the sample sizes required were previously calculated in order to detect a given improvement in FBF. A sample of eight patients is sufficient to detect a 20% improvement in FBF, a sample of 12 patients is sufficient to detect a 15% improvement in FBF with a power of 90% and $\alpha = 0.05$. Differences between means were compared by paired and unpaired Student's *t*-test, as appropriate. The responses to SNP, ACh, L-NMMA and vitamin C were compared by ANOVA for repeated measurements and, when analysis was significant, Tukey's test was applied. Reproducibility of FBF and VR measurements were tested by using Pearson's coefficient. All calculated *P* values are

two tailed. Significant differences were assumed to be present at $P < 0.05$. All group data are reported as mean \pm S.D.

3. Results

Baseline demographic, hemodynamic and humoral characteristics are summarized in Table 1. According to inclusion criteria, serum cholesterol values were significantly lower in controls in comparison with hypercholesterolemics (4.5 ± 0.8 mmol/l vs 7.1 ± 0.9 mmol/l; $P < 0.0001$).

All participants were never smokers and no women were in postmenopausal status or taking estrogen replacement.

3.1. Reproducibility of endothelium-dependent vasodilation

The FBF and VR values, reevaluated in control subjects 2 months later and correlation coefficient are reported in Table 2. Similarly, the BP and heart rate (HR) did not change either.

3.2. Control subjects versus hypercholesterolemic patients

The basal FBF and forearm VR were not different in normal subjects and hypercholesterolemic patients; FBF mean values were: 3.6 ± 0.4 and 3.5 ± 0.6 ml·100 ml tissue⁻¹·min⁻¹ ($P = \text{NS}$), respectively; VR mean values were 26.1 ± 4.5 and 27.2 ± 5.6 U ($P = \text{NS}$), respectively.

Table 1
Baseline demographic, hemodynamic and humoral characteristics

	Controls (<i>n</i> = 12)	Hypercholesterolemics (<i>n</i> = 18)	
		Baseline	Atorvastatin
Age, y	39 \pm 5	40 \pm 6	
Gender, M/F	7/5	8/10	
Body mass index, kg/m ²	25.0 \pm 4.2	25.5 \pm 3.5	25.4 \pm 4.0
Heart rate, bpm	70.0 \pm 3.0	70.8 \pm 2.4	70.1 \pm 2.5
Total cholesterol, mmol/l	4.5 \pm 0.8	7.1 \pm 0.9 [†]	4.8 \pm 0.7 [†]
LDL-cholesterol, mmol/l	2.6 \pm 0.3	5.0 \pm 0.5 [†]	3.3 \pm 0.3 [†]
HDL-cholesterol, mmol/l	1.4 \pm 0.3	1.2 \pm 0.1*	1.4 \pm 0.2 [†]
Triglyceride, mmol/l	1.3 \pm 0.4	2.1 \pm 0.2 [†]	1.6 \pm 0.3 [†]
Glucose, mmol/l	5.0 \pm 0.2	5.1 \pm 0.4	5.0 \pm 0.5
Blood pressure, mm Hg	125/76 \pm 6/5	125/74 \pm 7/6	126/75 \pm 5/6
FBF ^a , ml·100 ml tissue ⁻¹ ·min ⁻¹	3.6 \pm 0.4	3.5 \pm 0.6	3.6 \pm 0.8
Vascular resistance, U	26.1 \pm 4.5	27.2 \pm 5.6	27.1 \pm 4.3

^a forearm blood flow.

* $P < 0.05$ hypercholesterolemics versus controls.

[†] $P < 0.0001$ hypercholesterolemics versus controls and atorvastatin versus hypercholesterolemics.

Table 2
Forearm blood flow (FBF) and vascular resistance (VR) in 12 normal volunteers at baseline and 2 months later

	Baseline	2 months	<i>r</i>
<i>FBF (ml·100 ml·tissue⁻¹·min⁻¹)</i>			
Basal	3.6 ± 0.4	3.5 ± 0.4	0.961*
ACh (30 µg ml ⁻¹ min ⁻¹)	27.0 ± 3.4	27.2 ± 3.7	0.895*
SNP (3.2 µg ml ⁻¹ min ⁻¹)	11.8 ± 0.7	11.9 ± 0.7	0.943*
<i>VR (U)</i>			
Basal	26.1 ± 4.5	26.4 ± 4.7	0.985*
ACh (30 µg ml ⁻¹ min ⁻¹)	3.2 ± 0.5	3.2 ± 0.6	0.987*
SNP (3.2 µg ml ⁻¹ min ⁻¹)	7.8 ± 2.9	7.6 ± 2.8	0.961*

* $P < 0.0001$.

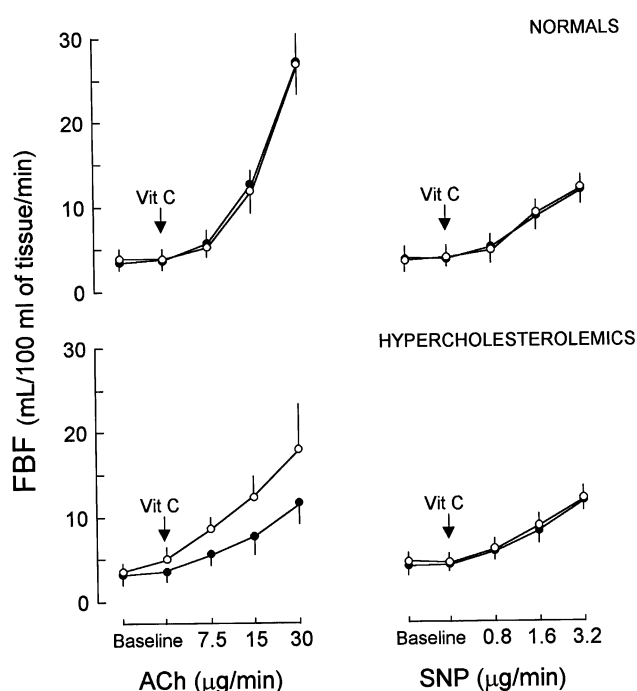


Fig. 1. Forearm blood flow (FBF) dose-response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) in normal control subjects and hypercholesterolemic patients during saline (●) and vitamin C (○) infusion are reported. The ACh-stimulated FBF is significantly attenuated in hypercholesterolemic ($P < 0.0001$ by ANOVA), but significantly improved ($P < 0.0001$ by ANOVA) by vitamin C coinfusion. Data are shown as mean \pm S.D. and expressed as absolute values. Non significant differences were found during SNP infusions.

3.2.1. Endothelium-dependent Vasodilation

The forearm ACh-stimulated vasodilation was significantly ($P < 0.0001$ by ANOVA) attenuated in hypercholesterolemic compared with controls (Fig. 1). The increments from basal in hypercholesterolemic and control subjects were 1.9 ± 1.2 , 4.2 ± 2.1 , 8.0 ± 1.2 ml·100 ml tissue⁻¹·min⁻¹ and 2.1 ± 1.5 , 9.3 ± 5.1 , 23.4 ± 6.3 ml·100 ml tissue⁻¹·min⁻¹, respectively. At

the highest dose of ACh (30 µg/min), FBF increased to 11.5 ± 1.9 ml·100 ml tissue⁻¹·min⁻¹ in hypercholesterolemic compared with 27.0 ± 3.4 ml·100 ml tissue⁻¹·min⁻¹ in normal subjects ($P < 0.0001$).

Forearm VR during ACh infusions significantly decreased in hypercholesterolemic (decrements: 9.6 ± 2.3 , 15.2 ± 3.7 , 19.0 ± 4.7 U) and in controls (decrements: 9.2 ± 3.4 , 16.2 ± 7.4 , 22.9 ± 7.3 U). At the highest dose of ACh (30 µg/min), VR was 3.2 ± 0.5 U in controls and 8.2 ± 2.0 U ($P < 0.0001$) in hypercholesterolemic.

FBF and VR in the contralateral arm did not change during incremental ACh infusions in either group of subjects. Similarly, ACh infusions did not affect BP or HR.

3.2.2. Endothelium-independent vasodilation

Significant increases in FBF as well as decreases in forearm VR were observed in both normal subjects and hypercholesterolemic during SNP infusions, though no significant differences were found between groups (Fig. 1). At the highest dose (3.2 µg/min), FBF increased to 11.8 ± 0.7 ml·100 ml tissue⁻¹·min⁻¹ in the control group and to 11.6 ± 0.8 ml·100 ml tissue⁻¹·min⁻¹ in hypercholesterolemic, respectively ($P = \text{NS}$). At this dose, the forearm VR decreased to 7.8 ± 2.9 U in control group compared with 7.9 ± 2.1 U in hypercholesterolemic ($P = \text{NS}$).

SNP infusions did not affect FBF and VR in the contralateral arm and did not change BP or HR.

3.3. Vitamin C administration

Intra-arterial infusion of vitamin C significantly increased basal FBF in hypercholesterolemic from 3.5 ± 0.9 to 4.7 ± 0.9 ml·100 ml tissue⁻¹·min⁻¹ ($P < 0.0001$); no significant differences were observed in control group, 3.6 ± 0.6 – 3.7 ± 0.9 ml·100 ml tissue⁻¹·min⁻¹ ($P = \text{NS}$). Basal VR in hypercholesterolemic decreased from 27.7 ± 6.2 to 20.4 ± 4.2 U ($P < 0.0001$), but was unchanged in normal subjects, 25.5 ± 4.1 to 24.9 ± 4.7 U ($P = \text{NS}$).

3.3.1. Endothelium-dependent vasodilation

The vasodilator response to ACh in hypercholesterolemic significantly increased during co-administration of vitamin C ($P < 0.0001$ by ANOVA) (Fig. 1). At the highest dose of ACh (30 µg/min) plus vitamin C (24 mg/min), the FBF increased to 18.0 ± 5.6 ml·100 ml tissue⁻¹·min⁻¹ ($P < 0.0001$); forearm VR decreased to 5.7 ± 1.8 U ($P < 0.0001$).

On the contrary, in control group the ACh-stimulated vasodilation was not significantly affected by co-administration of vitamin C (Fig. 1). At the highest dose of ACh (30 µg/min), before and during co-infusion of vitamin C (24 mg/min), the FBF was 27.0 ± 3.4 ml·100 ml tissue⁻¹·min⁻¹ and 27.1 ± 4.0 ml·100 ml

tissue⁻¹·min⁻¹ ($P = \text{NS}$), respectively. At this dose, VR was unaffected by co-infusion of vitamin C, 26.1 ± 2.5 to 25.9 ± 3.2 U ($P = \text{NS}$).

3.3.2. Endothelium-independent vasodilation

In both normal subjects and in hypercholesterolemics, the vasodilator response to SNP was not significantly modified during co-administration of vitamin C (Fig. 1). In hypercholesterolemics, at the highest dose of SNP (3.2 $\mu\text{g}/\text{min}$), the FBF was 11.6 ± 0.8 ml·100 ml tissue⁻¹·min⁻¹ before and 11.7 ± 0.9 ml·100 ml tissue⁻¹·min⁻¹ during co-infusion of vitamin C ($P = \text{NS}$). At this dose, the forearm VR before and during

co-administration of vitamin C was 7.8 ± 1.9 and 7.7 ± 2.1 U ($P = \text{NS}$), respectively.

3.4. Atorvastatin treatment

3.4.1. Effects on lipid profile and hemodynamic parameters

Atorvastatin treatment significantly lowered both serum cholesterol and triglyceride values. After 4 weeks, in comparison with placebo, total cholesterol was 32% lower ($P < 0.0001$), LDL-cholesterol was 34% lower ($P < 0.0001$), triglyceride were 24% lower ($P < 0.0001$) and HDL-cholesterol was 17% higher ($P < 0.0001$).

The basal FBF and VR were not different between atorvastatin and placebo treatment periods: 3.6 ± 0.8 versus 3.5 ± 0.9 ml·100 ml tissue⁻¹·min⁻¹ ($P = \text{NS}$) and 27.1 ± 4.3 versus 27.3 ± 3.4 U ($P = \text{NS}$), respectively.

3.4.2. Effects on ACh-vasodilation

The ACh-mediated vasodilation significantly increased between placebo and 4 weeks ($P < 0.0001$ by ANOVA) of atorvastatin treatment. At the highest dose (30 $\mu\text{g}/\text{min}$), FBF significantly ($P < 0.0001$ by ANOVA) increased to 14.9 ± 1.5 ml·100 ml tissue⁻¹·min⁻¹ (+313.8%) (Fig. 2). At this dose, forearm VR significantly decreased to 7.2 ± 1.6 U ($P < 0.0001$). However, it is necessary to remark that even if atorvastatin treatment markedly improved the FBF response to ACh, it still remained depressed in hypercholesterolemics when compared with control subjects.

The ACh-stimulated FBF and VR mean values after four weeks of placebo treatment were not significantly different when compared to basal study. At the highest dose of ACh, the FBF and forearm VR were: 11.7 ± 2.1 ml·100 ml tissue⁻¹·min⁻¹ and 8.1 ± 2.2 U ($P = \text{NS}$). Thus, hypercholesterolemic patients who had previously received given to atorvastatin treatment, did not show a residual effect on endothelial function 4 weeks into placebo.

The ACh-stimulated vasodilation significantly increased during co-infusion of vitamin C ($P < 0.0001$ by ANOVA). At the maximal dose of ACh (30 $\mu\text{g}/\text{min}$) plus vitamin C (24 mg/min), FBF raised from 4.8 ± 0.8 to 18.7 ± 4.2 ml·100 ml tissue⁻¹·min⁻¹ (+289.5%) (Fig. 2). At this dose, forearm VR decreased to 5.1 ± 1.2 U. Thus, the peak percent increase in FBF after atorvastatin treatment and during vitamin C co-infusion were not significantly different.

3.4.3. Effects on SNP-vasodilation

Significant increase on FBF as well as decrease on VR were observed during SNP infusions in hypercholesterolemic patients after lipid lowering treatment. However, no significant differences with placebo group were found at the different time points. Similarly, responses to endothelium-independent vasodilator SNP were not

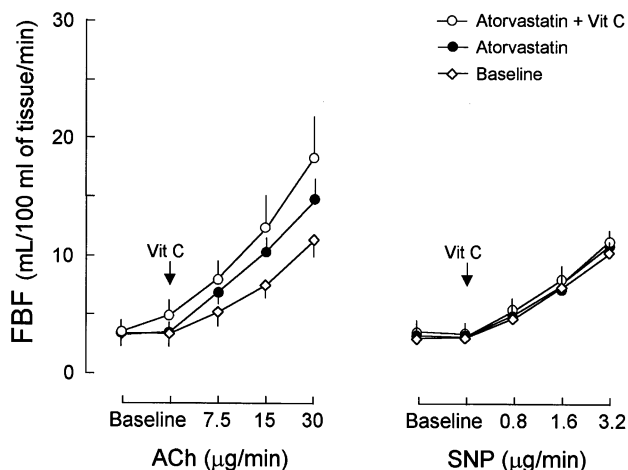


Fig. 2. Forearm blood flow (FBF) dose-response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) in hypercholesterolemic patients in baseline (\diamond), after 4 weeks of atorvastatin treatment (\bullet) and plus vitamin C co-infusion (\circ). Data are shown as mean \pm S.D. and expressed as absolute values.

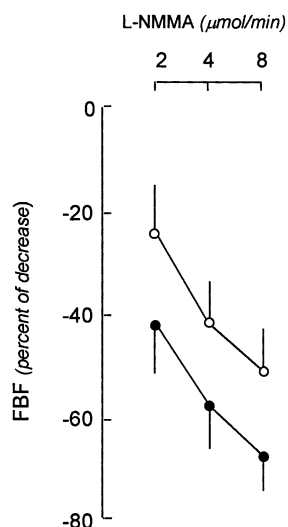


Fig. 3. Forearm blood flow (FBF) dose-response curves to L-NMMA in hypercholesterolemics at baseline (\circ) and after 4 weeks of atorvastatin treatment (\bullet). The vasoconstrictor effects of L-NMMA significantly increased after atorvastatin ($P < 0.0001$), indicating of an increased basal nitric oxide.

significantly modified by co-infusion of vitamin C (Fig. 2).

3.4.4. Effects on L-NMMA infusion

After 4 weeks of atorvastatin treatment the decrease in FBF induced by L-NMMA infusions was significantly greater than at baseline conditions, as reported in Fig. 3.

4. Discussion

Results obtained in this study confirm in hypercholesterolemics that: (1) endothelium-dependent vasodilation is blunted compared with normal subjects; (2) intra-arterial infusion of vitamin C, an antioxidant compound, improves the impaired ACh-mediated vasodilation; (3) atorvastatin is very effective in lowering rapidly serum cholesterol and triglyceride.

Moreover, after 4 weeks of atorvastatin treatment, our results provide the first evidence that ACh-stimulated vasodilation was significantly increased, as was L-NMMA-mediated vasoconstriction, indicating that both stimulated and basal endothelium-dependent vasodilation were improved. These data are consistent with those of Simons et al. [27] who in a previous study demonstrated that atorvastatin treatment in patients with severe primary hypercholesterolemia improved flow-mediated endothelium-dependent dilation in response to reactive hyperaemia. However, it is necessary to remark that the vascular response to hyperaemia may be influenced by different stimuli and, therefore, did not test the ACh-only stimulated vasodilation, as performed in our study. In agreement with data on simvastatin and vascular function reported by O'Driscoll [20], our study also demonstrates, for the first time, that L-NMMA-mediated vasoconstriction is enhanced after atorvastatin treatment, indicative of an increased basal effect of NO. The disagreement with Stroes [19] who reported unchanged L-NMMA responses after combined simvastatin and cholestyramine therapy, may be explained by differences in patients' characteristics, study design and analysis as, reported by O'Driscoll also [20].

4.1. Nitric oxide and atherosclerosis

NO is a major factor involved in the antiatherosclerotic properties of the normal endothelium. In fact, NO interferes with key events in the appearance and progression of atherosclerosis, such as monocytes and leukocytes adhesion as well as platelet-vessel wall interaction [32,33]. NO also reduces vascular tone and decreases endothelial permeability [34]; finally, it inhibits vascular smooth muscle cells migration and proliferation in vitro as well as in vivo [35]. Major risk factors for atherosclerotic vascular disease, such as hypertension, smoking, diabetes

and hypercholesterolemia have been associated with impaired NO activity [10–15].

4.2. Hypercholesterolemia and vascular function

The vascular response to muscarinic agonists depends on the integrity of the endothelial cells and the L-arginine-NO pathway. A depressed ACh-stimulated vasodilation was reported in animals with experimental hypercholesterolemia [36] and in the coronary and forearm vasculature of hypercholesterolemic humans [15,17]. This endothelial dysfunction can be attributed to decreased bioavailability of NO due to either decreased synthesis by endothelial cells or increased degradation by vascular oxidative stress. Recent experimental data support that the reduced NO bioavailability is more likely to be secondary to increased degradation by oxygen-derived free radicals rather than decreased production by endothelial cells.

In agreement with previous reports [12,15,17,19,20], the ACh-stimulated response, but not that for SNP, was depressed in our hypercholesterolemics compared with control group, confirming the presence of endothelial dysfunction in hypercholesterolemia. However, the depressed vasodilation was significantly improved by intra-arterial infusion of vitamin C; this effect of oxygen-free radical scavenger is probably specific because it was obtained neither in normal subjects nor on endothelium-independent vasodilation. Thus, these findings suggest that superoxide anion production in hypercholesterolemia accounts for endothelial dysfunction and indicate that vitamin C improves endothelial function, probably by direct scavenging oxygen-free radicals.

Vitamin C may also improve ACh-stimulated vasodilation by sparing from oxidative degradation intracellular glutathione that represents an important source of intracellular reduced thiols. An intracellular depletion of reduced thiols affects NO synthase activity in cultured endothelial cells [37].

4.3. Atorvastatin and endothelial function

In this randomized, controlled study, we have demonstrated that forearm endothelial dysfunction can be significantly improved by atorvastatin treatment. An important aspect of this study is the demonstration that vascular changes occur rapidly; the possible explanation is that improvement of endothelial function might not be due solely to a decrease in serum cholesterol. In fact, this possibility is supported by experimental data indicating that statins can interfere with some mechanisms involved in the atherosclerotic process, independently of their hypocholesterolemic properties [38]. Finally, our data support the possibility that atorvastatin may be acting as an antioxidant, which is consistent with action exerted by simvastatin in human monocyte-derived macrophages

[39] and with data recently published by Aviram [40]. In fact, in our study peak percent increase from baseline in ACh-stimulated vasodilation after atorvastatin treatment and during co-infusion of vitamin C was similar (313.8 vs 289.5%). Thus, because the potentiation of atorvastatin in ACh-induced vasodilation is similar to that exerted by vitamin C co-infusion and no additive effect was detected, oxidative stress may account, at least partly, for endothelial dysfunction in human hypercholesterolemia.

Another interesting evidence provided by our study is that lipid-lowering therapy improves, but does not completely normalize the endothelial response to ACh, suggesting that the restoration of normal endothelium-mediated vasodilation may probably require even more extended therapy, as has been reported in animal [41] and human studies [20].

4.4. Clinical implications

Controlled clinical studies of the statins demonstrate the significant benefit of these drugs in reducing CAD risk in hypercholesterolemics. Plaque rupture is a mechanical event within the vessel and it is integral to many pathophysiologic processes in vascular disease, including acute myocardial infarction, stroke and other acute vascular syndromes. Although the exact mechanism of action of plaque stabilization in response to cholesterol-lowering treatment is still unclear, improvement of endothelial function represents a critical component. The reduction of vascular tone and structural stabilization of atherosclerotic plaques, undetectable on angiogram, may effect a reduction in vascular events rate. Therefore, because elevated cholesterol levels induce deleterious effects on the lipid core composition of the plaque, an increase in mechanical load and a decrease in endothelial function, it is to be hoped that clinicians should institute lipid-lowering therapy in all patients with clinical or preclinical manifestation of atherosclerotic vascular disease.

5. Conclusions

This study provides the first evidence that atorvastatin rapidly improves but does not normalize the ACh-stimulated vasodilation in hypercholesterolemics. The study also demonstrates that superoxide anion production accounts for this endothelial dysfunction and indicates that vitamin C induces a significant improvement on endothelium-dependent vasodilation, probably by direct scavenging oxygen-free radicals. Our data are consistent with the proposed antioxidant action of statins because percent peak increase in ACh-stimulated FBF after atorvastatin treatment and

during co-infusion of vitamin C are similar. Thus, the beneficial effects exerted by atorvastatin treatment on hypercholesterolemics may be explained by lipid-lowering action and also by the recently proposed pleiotropic effects, including the improvement of endothelial dysfunction that represents the earlier atherosclerotic lesion.

Acknowledgements

We'll be forever grateful to Prof. Mattioli, our unforgettable magister, for his lessons and affection.

References

- [1] Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990;323:27–36.
- [2] Furchgott RF, Zawadzki JV. The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373–6.
- [3] Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;327:524–6.
- [4] Lüscher TF, Vanhoutte PM. The Endothelium: Modulator of Cardiovascular Function. Boca Raton, FL: CRC Press, 1990.
- [5] Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS. Modulation of vascular smooth muscle contraction by the endothelium. *Ann Rev Physiol* 1986;48:307–20.
- [6] Smith RP, Kruszyna H. Nitroprusside produces cyanide poisoning via reaction with hemoglobin. *J Pharmacol Exp Ther* 1974;191:557–63.
- [7] Lüscher TF, Cooke JP, Houston DS, Neves RJ, Vanhoutte PM. Endothelium-dependent relaxations in human arteries. *Mayo Clin Proc* 1987;62:601–6.
- [8] Thom S, Hughes A, Martin G, Sever PS. Endothelium-dependent relaxation in isolated human arteries and veins. *Clin Sci* 1987;73:547–52.
- [9] Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989;2:997–1000.
- [10] Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22–7.
- [11] Linder L, Kiowski W, Bühler FR, Lüscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in essential hypertension. *Circulation* 1990;81:1762–7.
- [12] Zeithner AM, Drexler H, Saubier B, Just H. Endothelium-mediated coronary blood flow modulation in humans: effects of age, atherosclerosis, hypercholesterolaemia and hypertension. *J Clin Invest* 1993;92:652–62.
- [13] Zeithner AM, Schachinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilation function. *Circulation* 1995;92:1094–100.
- [14] Johnstone MT, Creager SJ, Scales KM, et al. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993;88:2510–6.
- [15] Creager MA, Cooke JP, Mendelson ME, et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990;86:228–34.

- [16] Ting HH, Timini FK, Haley EA, et al. Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. *Circulation* 1997;95:2617–22.
- [17] Anderson TJ, Meredith IT, Yeung AC. The effect of cholesterol-lowering and anti-oxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med* 1995;332:488–93.
- [18] McCrohon JA, Adams MR, McCredie RJ, et al. Hormone replacement therapy is associated with improved arterial physiology in healthy postmenopausal women. *Clin Endocrinol* 1996;45:435–41.
- [19] Stroes ESG, Koomans HA, de Bruin TWA, Rabelink TJ. Vascular function in the forearm of hypercholesterolemic patients off and on lipid-lowering medication. *Lancet* 1995;346:467–71.
- [20] O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-Coenzyme A reductase inhibitor, improve endothelial function in 1 month. *Circulation* 1997;95:1126–31.
- [21] Mancini GBJ, Hanry GC, Macaya C, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 1996;94:258–65.
- [22] Taddei S, Virdis A, Ghiadoni L, et al. Lacidipine restores endothelium-dependent vasodilation in essential hypertensive patients. *Hypertension* 1997;30:1606–12.
- [23] Perticone F, Ceravolo R, Mario R, et al. Calcium antagonist isradipine improves abnormal endothelium-dependent vasodilation in never treated hypertensive patients. *Cardiovasc Res* 1999;41:299–306.
- [24] Scandinavian Simvastatin Survival Study: Investigators Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383–1389.
- [25] Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia: West of Scotland Coronary Prevention Study. *N Engl J Med* 1995;333:1301–7.
- [26] Alanpovic P, Heinonen T, Shurzinske L, Black DM. Effect of a new HMG-CoA reductase inhibitor, atorvastatin, on lipids, apolipoproteins and lipoprotein particles in patients with elevated serum cholesterol and triglyceride levels. *Atherosclerosis* 1997;133:123–33.
- [27] Simons LA, Sullivan D, Simons J, Celermajer DS. Effects of atorvastatin monotherapy and simvastatin plus cholestyramine on arterial endothelial function in patients with severe primary hypercholesterolemia. *Atherosclerosis* 1998;137:197–203.
- [28] Perticone F, Ceravolo R, Maio R, et al. Angiotensin-converting enzyme gene polymorphism is associated with endothelium-dependent vasodilation in never treated hypertensive patients. *Hypertension* 1998;31:900–5.
- [29] Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 1996;94:6–9.
- [30] Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 1998;97:2222–9.
- [31] Timini FK, Ting HH, Haley EA, et al. Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1998;31:552–7.
- [32] Gauthier TW, Scalia R, Murohara T, Guo JP, Lefer AM. Nitric oxide protects against leukocyte-endothelium interactions in the early stages of hypercholesterolemia. *Arterioscl Thromb Vasc Biol* 1995;15:1652–9.
- [33] de Graaf JC, Banga JD, Moncada S, et al. Nitric oxide inhibitor of platelet adhesion under flow conditions. *Circulation* 1992;85:2284–90.
- [34] Draaijer R, Atsma DE, van der Laarse A, van Hinsbergh VW. cGMP and nitric oxide modulate thrombin-induced endothelial permeability: regulation via different pathways in human aortic and umbilical vein endothelial cells. *Circ Res* 1995;76:199–208.
- [35] Sarkar R, Webb RC, Stanley JC. Nitric oxide inhibition of endothelial mitogenesis and proliferation. *Surgery* 1995;118:274–9.
- [36] Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M. Low density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. *Nature* 1987;327:237–9.
- [37] Murphy ME, Piper HM, Watanabe H, Sies H. Nitric oxide production by cultured aortic endothelial cells in response to thiol depletion and replenishment. *J Biol Chem* 1991;266:19378–83.
- [38] Gellmann J, Ezekowitz MD, Sarembock IJ, et al. Effect of lovastatin on intima hyperplasia after balloon angioplasty. A study in an atherosclerotic hypercholesterolemic rabbit. *J Am Coll Cardiol* 1991;17:251–9.
- [39] Giroux LM, Davignon J, Naruszewicz M. Simvastatin inhibits the oxidation of low-density lipoproteins by activated human macrophages. *Biochim Biophys Acta* 1993;1165:335–8.
- [40] Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. *Atherosclerosis* 1998;138:271–80.
- [41] Harrison DG, Armstrong ML, Freiman PC, Heistad DD. Restoration of endothelium-dependent relaxation by dietary treatment of atherosclerosis. *J Clin Invest* 1987;80:1808–11.