

Fluvastatin therapy improves microcirculation in patients with hyperlipidaemia

Eva Haak^a, Claudia Abletshauser^c, Sonja Weber^a, Christine Goedicke^a,
Nicole Martin^a, Notbert Hermanns^b, Karl Lackner^d, Klaus Kusterer^a,
Klaus Henning Usadel^a, Thomas Haak^{b,*}

^a Medical Department I, Center of Internal Medicine, University-Hospital, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany

^b Research Institute of the Diabetes Academy Mergentheim, Theodor-Klotzbücher-Str. 12, D-97980 Bad Mergentheim, Germany

^c Novartis Pharma, Clinical Research, Nürnberg, Germany

^d Institute for Clinical Chemistry and Laboratory Medicine, University-Hospital, Regensburg, Germany

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Abstract

The purpose of this study was to investigate the effect of fluvastatin on the microcirculation of patients with hyperlipidaemia (low-density lipoprotein cholesterol >160 mg/dL, triglycerides <350 mg/dl) inadequately controlled by diet. After a dietary run-in of 4 weeks, patients were randomised in a double-blind study to receive fluvastatin 40 mg twice daily ($n = 24$) or placebo ($n = 24$) for 12 weeks. The effect on microcirculation was assessed using capillary microscopy and laser Doppler fluxmetry at the nailfold at baseline and at 6 and 12 weeks after initiation of therapy. Capillaroscopy showed that fluvastatin improved microcirculation, i.e. time to peak flow during postocclusive reactive hyperaemia dropped from 19.7 ± 7.2 s at baseline to 12.3 ± 9.5 s at week 6 ($P < 0.01$) and 10.6 ± 6.5 s at week 12 ($P < 0.0001$). These results were confirmed using laser Doppler fluxmetry to study microcirculation in thermoregulatory capillaries at the same site. A significant decrease in total and LDL-cholesterol was achieved during fluvastatin therapy. In conclusion, fluvastatin therapy improves microcirculation in nutritive as well as thermoregulatory capillaries in hypercholesterolaemic patients within 6 weeks. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Hyperlipidaemia has long been known to be a risk factor for the development of atherosclerosis and hence increased cardiovascular morbidity and mortality. Endothelial dysfunction and the formation of atherosclerotic plaques on the vessel wall are two major processes that contribute to the pathophysiology of vascular disease [1,2]. In people with hyperlipidaemia with and without coronary artery disease (CAD) the endothelium has been shown to be functionally abnormal. This abnormality has been shown to exist before plaques

appear, and certainly before atherogenic lesions can be detected clinically [3].

Endothelial dysfunction is thought to be caused by modified, rather than native, low-density lipoprotein (LDL) cholesterol [4]. Clinically this translates into impaired vasodilation, or even paradoxical vasoconstriction, as shown with acetylcholine stimulation. In hypercholesterolaemic subjects, the major mechanism of impairment seems to be a diminished release of nitric oxide (NO) and/or an increased breakdown of NO [4–6]. The NO system also plays an important role in the late phase of arterial postocclusive reactive hyperaemia, a functional test that is used to determine the perfusion reserve on demand [7–9].

Fluvastatin is a semisynthetic statin which is effective in lowering lipid levels in a wide range of patient populations [10–12] and also has shown anti-athero-

* Corresponding author. Tel.: +49-7931-594101; fax: +49-7931101.

E-mail address: haak@diabetes-zentrum.de (T. Haak).

genic, antithrombotic and antioxidant properties [13,40]. The cholesterol-lowering effect of fluvastatin is associated with improvements in endothelium-dependent vasodilation. Treatment with fluvastatin resulted in a substantial increase in forearm bloodflow in response to reactive hyperaemia, as well as in response to acetylcholine in hypercholesterolaemic patients [14,15]. Several other studies with lipid-lowering drugs document the link between cholesterol lowering and improved vascular function in hypercholesterolaemia [16–21].

Recent experimental and clinical studies have clearly demonstrated that abnormalities of endothelium-dependent relaxation in atherosclerosis extend to the microvasculature, e.g. of the cardiac tissue, even though the corresponding macrovascular sections are free of overt atherosclerosis [5,22,23]. However, there are only few data concerning the efficacy of lipid-lowering therapy on peripheral microcirculation [24–27].

Thus, this study was designed to determine whether treatment of hyperlipidaemia with the statin fluvastatin was effective in improving peripheral microcirculation, and how soon after treatment was started could any beneficial effects be detected. Since high plasma levels of homocysteine [28,29] and an increase in procoagulant activity are also risk factors for CAD, effects on these parameters have been investigated in parallel.

2. Methods

The investigation was carried out according to the guidelines of Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved by the local ethics committee. Before the patients were enrolled into the study, written informed consent was obtained from each participant.

2.1. Patients

This study recruited patients with hyperlipidaemia (Low density lipoprotein (LDL) cholesterol > 160 mg/dl, triglycerides < 350 mg/dl) who were inadequately controlled by diet.

2.2. Study protocol

Patients were asked to follow a standardised low-fat diet for 4 weeks before the start of the study, in accordance with the guidelines of the European Atherosclerosis Society [30]. Patients were then randomised to receive either fluvastatin 40 mg twice daily or placebo. The following parameters were evaluated at baseline, 6 and 12 weeks: microcirculation assessed by combined video capillaroscopy and laser Doppler flux, as well as laser Doppler image scan; lipid status [total,

LDL- and high-density lipoprotein (HDL)-cholesterol and triglycerides]; levels of apolipoprotein B, homocysteine and the blood coagulation factors fibrinogen, factor VII, antithrombin III, von Willebrand factor, prothrombin fragments I + II, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (t-PA) and thrombomodulin.

2.3. Safety

Safety was assessed by clinical examination, monitoring of laboratory parameters (including creatinine, creatine kinase, gamma-glutamyl transpeptidase, serum glutamic oxalo-acetic transaminase, serum glutamic pyruvic transaminase) and vital signs and reporting of adverse events.

2.4. Assessment of microcirculation

Patients were required to refrain from smoking and from drinking caffeinated beverages for at least 12 h prior to the investigation in order to exclude artificial vasoactive effects [31,32]. All investigations were performed in the morning with patients sitting in an upright position. During the investigations the patient's hand rested at heart level in a standardised position. Skin temperature was continuously monitored with a digital-electronic thermistor (Digimed H 11, ttw, Waldkirch, Germany) and maintained in the range 27.3–30.5°C to avoid temperature-related changes in microcirculation [33]. The room temperature was maintained at 21–24°C.

Microcirculation was studied at rest and in a dynamic test using nailfold capillaroscopy on the fourth finger of the left hand. This method was used because the capillaries extend parallel to the surface. Laser Doppler perfusion monitoring was performed at the bottom of the same finger in parallel as described below. For dynamic measurements, postocclusive reactive hyperaemia was used as a provocative test and microcirculation was assessed before and after 2 min of vessel occlusion (cuff inflated to 200 mmHg). Both methods have been evaluated in a large variety of clinical trials and proven to be reliable tools in the investigation of microcirculation [31,33–35]. The retest-reliability of video capillaroscopy and laser Doppler perfusion monitoring was assessed and has been proved to be sufficient before in various studies by calculating the intra-class correlation coefficients in the placebo group according to the determination of the Pearson coefficient and the methods described by Bland and Altman [36]. In addition a laser Doppler image scan of the whole back of the left hand was performed before the dynamic test in order to avoid accidental differences due to single measurements close to arteries or veins.

2.5. Video capillaroscopy

An appropriate capillary was selected using a 6.3/0.20 objective lens with a 150-fold magnification and capillary blood cell flow was recorded with a 20/0.4 oil objective, resulting in a 1500-fold magnification of the capillary bed overall. The same capillary was used for subsequent measurements. Each investigation was video-recorded for the subsequent analysis. Microcirculation in the nutritional capillaries was measured with the aid of a computerised analysis system using temporal cross-correlation (CapiFlow[®], Lawrenz Electronics, Sulzbach, Germany). This system calculates the blood cell velocity by the dual-window technique. The computer generates two photometric windows. The inter-window distance and the size of the windows are adjusted to the size of the different capillaries. From the interwindow distance and the passage time of blood cells through both windows, the system is able to calculate capillary blood cell velocity. Calculations were repeated six times per second and given as mean values of the single measurements. Artefacts, e.g. due to unintentional movements of the patient, are recognised and eliminated automatically. Although this method had been validated in our laboratory by manual frame-to-frame analysis prior to this study, manual analysis was used to spot check the computer-assisted analyses.

2.6. Laser Doppler flux

A laser Doppler imager (Moor LDI V 3.0, Moor Instruments Ltd, Devon, UK) capable of laser Doppler perfusion imaging and laser Doppler perfusion monitoring was used. A low-power laser beam was directed at the skin and the Doppler-shifted light from moving blood and the non-shifted light from tissue was detected by two square-law detectors. The signals were processed to give *flux* and *concentration* parameters proportional to tissue blood flow and the concentration of moving blood cells, respectively. The laser Doppler was calibrated using a standard flux signal from polystyrene microspheres undergoing thermal (Brownian) motion. The laser Doppler perfusion monitoring was performed by measuring laser Doppler flux on the same skin point as used in a dynamic test. For the laser Doppler perfusion imaging (image scan), the laser light was directed via a moving mirror to execute a raster pattern across the back of the left hand. This gave the average perfusion for the defined area (RU) and avoided artefacts arising from positioning of the laser beam close to a large vessel for a single measurement. Blood pressure and heart rate were measured before each investigation.

Both capillaroscopy and laser Doppler perfusion monitoring were used to measure capillary blood cell velocity at rest (rCBV) and the time to peak capillary

blood cell velocity (tpCBV) during postocclusive reactive hyperaemia.

2.7. Determination of lipid and haemostasiologic parameters

Levels of total cholesterol and triglycerides were measured enzymatically. HDL- and LDL-cholesterol were measured after precipitation with phosphorous-wolfram-acid and polyvinylsulphate, respectively. Apolipoprotein B was measured using an immunonephelometric test. Assays for fibrinogen, factor VII and antithrombin III were performed with reagents from Dade-Behring (Marburg, Germany) on a Behring Coagulation System. Von Willebrand factor activity was determined by agglutination of stabilised platelets in the presence of ristocetin A (Dade-Behring).

Tissue plasminogen activator and prothrombin fragments I + II were determined by ELISA (Dade-Behring and Roche-Diagnostics, respectively). PAI-1 activity was analysed by a chromogenic assay which measured the cleavage of a chromogenic substrate by urokinase-activated plasmin.

Homocysteine was treated with ammonium-7-fluorobenzo-2-oxa-1,3-diazol-4-sulfonate (SBD-F, Wako Chemicals, Düsseldorf, Germany) and the fluorescent product was quantified by HPLC with fluorimetric detection.

2.8. Statistical analysis

Data are given as arithmetic mean \pm standard deviation of the mean. Descriptive statistics were performed on all parameters. The primary efficacy parameter was the tpCBV during postocclusive reactive hyperaemia. Differences within and between treatment groups were tested for using the Wilcoxon 2-sample rank-test for paired and unpaired data. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

Sixty-four patients were randomised into the study. Four patients receiving fluvastatin discontinued therapy: three due to poor compliance, and one due to severe adverse events (a rise in serum liver enzymes, nausea, vomiting, gastro-intestinal pain and generally poor condition). One patient on placebo also discontinued due to an adverse event (nausea, vomiting). Fluvastatin was well tolerated and there were no clinically significant changes between the treated and placebo group in adverse-event profiles, laboratory values or vital signs. The present paper reports the results of patients who had a baseline and a final measurement (week 12) of capillaroscopy and laser Doppler flux. 48

Table 1
Serum lipid levels before and after 6 and 12 weeks' treatment with fluvastatin or placebo

Parameter (mg/dl)	Fluvastatin (n = 24)			Placebo (n = 24)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Total cholesterol	293 ± 42	203 ± 34 ^a	226 ± 45 ^a	301 ± 39	297 ± 33	321 ± 52
LDL-cholesterol	211 ± 39	121 ± 34 ^a	140 ± 42 ^a	205 ± 24	203 ± 23	210 ± 37
HDL-cholesterol	55 ± 16	57 ± 17	57 ± 18	56 ± 17	54 ± 15	54 ± 16
Triglycerides	148 ± 45	106 ± 37 ^b	142 ± 48	208 ± 113	210 ± 82	271 ± 182
Apolipoprotein B	141 ± 17	—	104 ± 29 ^a	147 ± 25	—	153 ± 33

^a $P < 0.0001$ versus placebo.

^b $P < 0.05$ versus placebo. Values expressed as mean ± standard deviation.

patients (24 receiving fluvastatin and 24 receiving placebo) had complete data. Beside 5 discontinuations, the major reasons for missing data were technical problems. Mean age of the patients was 55 years, and about 50% were female. With respect to age, gender, lipid levels, and microcirculatory parameters, patients were well comparable at baseline.

3.1. Lipid status

Fluvastatin therapy resulted in decreases in total and LDL-cholesterol after 12 weeks of 22.8% and 33.6%, respectively. The decrease was significant compared with baseline at both 6 and 12 weeks. HDL-cholesterol and triglyceride levels remained unchanged. The results are given in Table 1. Furthermore, apolipoprotein B levels were statistically significantly lower ($p < 0.0001$) following 12 weeks' fluvastatin treatment compared with baseline and placebo treatment.

3.2. Microcirculation

Treatment with fluvastatin significantly improved microcirculation. In nutritional capillaries, compared with baseline the mean change in tpCBV with fluvastatin treatment was statistically significant at 6 weeks (-7.36 ± 10.96 s; $P < 0.005$) and 12 weeks (-9.04 ± 7.60 s; $P < 0.0001$) (Fig. 1a). The changes with placebo treatment were not statistically significant. Between-treatment comparison of the microcirculation in the nutritional capillaries showed that the proportional changes were significantly greater with fluvastatin compared with placebo at weeks 6 and 12 ($p < 0.05$, $P < 0.001$, respectively). In thermoregulatory capillaries the mean tpCBV was reduced following 6 and 12 weeks ($P < 0.05$) fluvastatin treatment (Fig. 1b). Furthermore, a correlation analysis between the final LDL-cholesterol level and the time to peak flow as assessed by capillaroscopy showed a positive correlation ($r = 0.37$; $P < 0.01$). Laser Doppler image scanning showed an increase in skin perfusion at rest from 16.8 to 19.2 at week 6 and to 18.3 (mean flux in RU) at week 12 under

fluvastatin, but this was not statistically significant compared with placebo.

Between treatment groups, resting capillary blood cell velocity, heart rate as well as systolic and diastolic blood pressure during video capillaroscopy and laser Doppler fluxmetry, respectively, did neither differ at baseline nor at week 12.

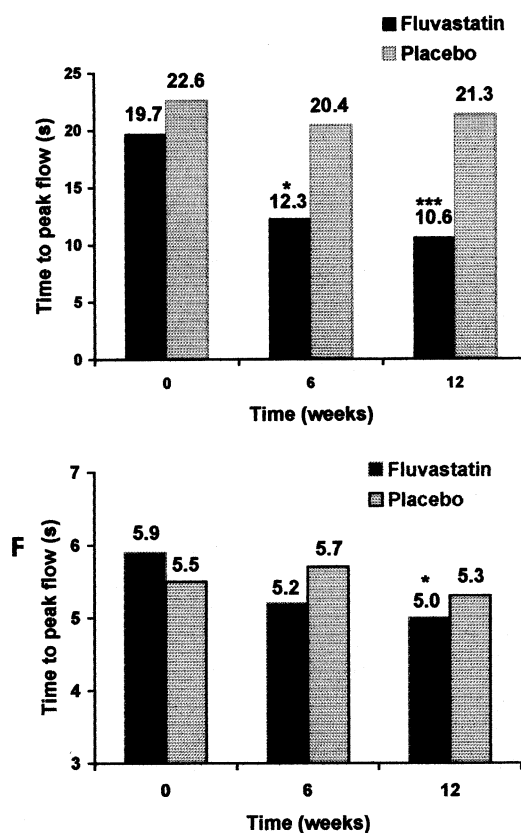


Fig. 1. Time to peak capillary blood cell velocity assessed by (a) capillary microscopy and (b) laser Doppler fluxmetry in patients at baseline and after 6 and 12 weeks' treatment with fluvastatin or placebo. * $P < 0.05$ between groups; *** $P < 0.001$ between groups.

Table 2
Parameters of blood coagulation and homocysteine levels in patients with hyperlipidaemia before and after 12 weeks' treatment with fluvastatin

	Fluvastatin (<i>n</i> = 31)		Placebo (<i>n</i> = 27)	
	Baseline	12 weeks	Baseline	12 weeks
Antithrombin III activity (%)	107.3 ± 13.0	105.8 ± 9.61 ^b	108.6 ± 14.3	117.5 ± 54.3
Factor VII (%)	115.6 ± 24.6	109.6 ± 19.1 ^b	119.2 ± 30.0	117.3 ± 30.1
Fibrinogen (mg/dL)	266.2 ± 78.7	294.3 ± 78.4	303.5 ± 63.5	299.1 ± 51.2
Homocysteine (μmol/L)	12.77 ± 2.81	12.61 ± 2.56	13.21 ± 3.95	13.06 ± 3.69
Plasminogen activator inhibitor (U/ml)	5.86 ± 13.42	3.57 ± 1.84	4.20 ± 1.96	4.40 ± 2.72
Fragments I+II (nmol/l)	1.60 ± 1.65	1.11 ± 0.38 ^a	1.24 ± 0.43	1.27 ± 0.51
Thrombomodulin (ng/ml)	4.97 ± 1.28	4.54 ± 1.02	4.99 ± 1.58	5.06 ± 1.57
D-dimer (ug/ml)	0.12 ± 0.10	0.12 ± 0.10	0.16 ± 0.10	0.16 ± 0.10
Tissue plasminogen activator (ng/ml)	7.64 ± 3.38	7.61 ± 3.57	7.41 ± 2.53	7.61 ± 3.0
von Willebrand factor (%)	121.5 ± 60.0	109.2 ± 60.1	116.6 ± 62.1	114.6 ± 61.6

^a *P* < 0.05 versus placebo and baseline.

^b *P* < 0.05 versus baseline; values expressed as mean ± standard deviation.

3.3. Coagulation factors and homocysteine

The levels of coagulation factors and homocysteine at baseline and last assessment for all patients who had a baseline and follow-up value are shown in Table 2. Levels of prothrombin fragments I + II, factor VII, and antithrombin III activity were statistically significantly lower (signed rank test) following 12 weeks' treatment with fluvastatin compared with baseline. Only the change in prothrombin fragments I + II was statistically significant versus placebo (*P* < 0.05).

4. Discussion

This double-blind, randomised, placebo-controlled study investigated the effect of lipid-lowering therapy with fluvastatin on peripheral microcirculation as assessed by tpCBV during postocclusive reactive hyperaemia. This is a sensitive test of capillary perfusion reserve (demand perfusion) and is known to reflect the impact of endothelial dysfunction on microcirculation [34]. Microcirculation was assessed by the complementary methods of capillary microscopy and laser Doppler fluxmetry at the same site. Capillary microscopy evaluates microcirculation in the nutritional capillaries (15% of the capillary bed) while laser Doppler fluxmetry does mostly the same for the subcapillary and thermoregulatory capillaries (85% of the capillary bed) [35].

The results clearly demonstrate that fluvastatin exerts a beneficial effect on microcirculation. After only 6 weeks' treatment there was a significant improvement in microvascular perfusion reserve seen as a reduced tpCBV, compared with placebo. This was confirmed after 12 weeks. Capillary microscopy showed the tpCBV decreased from 19.7 to 12.3 s at 6 weeks and 10.6 s at 12 weeks. The final value was only slightly higher than that reported for healthy controls (7.8 ± 2.4 s) [34]. This effect was also apparent with laser Doppler

fluxmetry, demonstrating that the benefits were not restricted to one compartment of the vasculature. The effects were accompanied by a reduction in total and LDL-cholesterol, as well as apolipoprotein B.

There is no doubt that lipid-lowering therapy improves endothelial function in the coronary as well as in the peripheral vascular system in hyperlipidaemic patients with and without CAD. Beside fluvastatin, clinical studies with e.g. lovastatin or simvastatin did elucidate mechanisms for prevention of atherosclerosis by restoration of endothelial function [1,18–21].

Beneficial effects of lipid-lowering therapy using either statins or fibrates on the peripheral microcirculation have been shown in a few reports in recent years [24–27]. More data are available on myocardial circulation. Fluvastatin improved myocardial microcirculation in patients with CAD, as assessed by positron emission tomography and thallium scintigraphy [37,38]. In an open study, laser Doppler fluxmetry showed that 24 weeks' therapy with fluvastatin (40 mg) improved the microcirculation in the forearm in patients with peripheral vascular disease and hypercholesterolaemia [25]. There are also a few studies indicating that fibrates also improve microcirculation in the forearm and nailfold [26,27]. Haemorrhological factors such as blood viscosity and erythrocyte deformability have an indirect effect on the microcirculation, and may be affected by blood cholesterol level [39,41]. Kohno et al. [24] showed that erythrocyte deformability was improved in parallel with cholesterol lowering after 1 year of pravastatin therapy. To the best of our knowledge, the present study is the first to investigate the effect of statin therapy on peripheral microcirculation in a double-blind, randomised, placebo-controlled trial.

The NO system is one of the most likely mechanisms by which fluvastatin improves the microcirculation. There is good clinical evidence that the release of NO is involved in the late phase of reactive hyperaemia in peripheral and coronary circulation [8,9]. In a recent

study, John et al. [15] showed that the improvement in endothelial-dependent vasodilation in response to fluvastatin therapy was indeed mediated by NO. Since endothelial dysfunction is believed to be mainly caused by oxidized LDL the effect of fluvastatin may be mediated both by a drop in LDL levels, which itself reduces the level of LDL oxidation, or directly by the antioxidant activity of fluvastatin. Furthermore, a recently published study showing a reduction in cardiac events under fluvastatin after one year of treatment supports the benefit for patients with severe atherosclerosis [41].

The novel findings of this investigation are that lipid lowering by fluvastatin improves peripheral microcirculation very rapidly, with significant benefits evident at 6 weeks. The present data support the concept that lipid-lowering treatment at an early stage, i.e. without manifest CAD, improves vascular function in hypercholesterolaemic patients.

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