Atherosclerosis 205 (2009) 227–232

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Flow-mediated dilation in patients with coronary artery disease is enhanced by high dose atorvastatin compared to combined low dose atorvastatin and ezetimibe: Results of the CEZAR study

Mir Abolfazl Ostad[a], Silke Eggeling[b], Peter Tschentscher[c], Edzard Schwedhelm[d], Rainer Böger[d], Philip Wenzel[a], Thomas Meinertz[b], Thomas Munzel[a], Ascan Warnholtz[a,*]

[a] Department of Medicine II, Johannes Gutenberg-University Mainz, Germany
[b] University Heart Center, Department of Cardiology, Hamburg, Germany
[c] University Hospital Hamburg-Eppendorf, Department of Clinical Chemistry, University of Hamburg, Germany
[d] University Hospital Hamburg-Eppendorf, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Hamburg, Germany

ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form 26 October 2008
Accepted 10 November 2008
Available online 9 December 2008

Keywords:
Brachial artery
Endothelium
Ezetimibe
Flow-mediated dilation
Statin

ABSTRACT

Background: Effects independent from cholesterol reduction on vascular function are considered to importantly contribute to the beneficial effects of statin therapy in cardiovascular disease. We aimed to evaluate the effect of high versus low dose atorvastatin on endothelial dysfunction in patients with coronary artery disease (CAD) in a setting of comparable cholesterol reduction.
Methods and results: Fifty-eight patients with CAD were randomly assigned to double-blind treatment for 8 weeks with atorvastatin 80 mg per day (A80) or atorvastatin 10 mg + ezetimibe 10 mg per day (A10E10), respectively. Flow-mediated vasodilation (FMD) of the brachial artery, nitroglycerin-mediated endothelium-independent vasodilation (NMD), lipid, C-reactive protein (CRP) plasma concentrations and urinary 8-iso-prostaglandin F2alpha excretion were measured before and after treatment. Total cholesterol, triglycerides and LDL-cholesterol levels were significantly reduced with no difference between A80 and A10E10. A80 caused significantly stronger improvement of FMD compared to A10E10 (absolute change FMD: A80 + 2.7 ± 3.0% (post vs. pre $p < 0.001$), A10E10 + 0.6 ± 2.9% (post vs. pre $p = 0.25$), A80 vs. A10E10 $p = 0.018$). NMD was improved by A80 but not by A10E10 (absolute change NMD: A80 + 2.7 ± 4.6%, A10E10 + 0.7 ± 3.5%, $p = 0.12$). Both treatment groups caused a comparable reduction of CRP and did not effect urinary 8-iso-prostaglandin F2alpha excretion. There was no correlation between FMD or NMD change and LDL-cholesterol change in either treatment group.
Conclusions: The present findings clearly suggest that in the presence of comparable LDL-lowering effects of both treatment forms, LDL-cholesterol independent effects of high dose atorvastatin therapy account for the improvement of endothelium-dependent vasodilation in patients with stable CAD.

Several large scale, randomized and controlled clinical trials have documented that cholesterol-lowering therapy with statins reduces the risk of death or cardiovascular events across a wide range of cholesterol levels in the setting of secondary [1], as well as primary coronary prevention [2]. Statin therapy therefore constitutes an integral part in the modern management of patients with coronary artery disease (CAD). There is accumulating evidence for various pleiotropic effects of statins on different cell types of the vasculature that could account for the beneficial effects even in the presence of low cholesterol levels. Some but not all of these proposed pleiotropic effects include an increase in endothelial NO-bioavailability, decrease in oxidative stress and LDL oxidation, enhanced stability of atherosclerotic plaques, inhibition of vascular smooth muscle cell proliferation and platelet aggregation, reduction of vascular inflammation and increase in circulating endothelial progenitor cells [3]. Randomized, controlled clinical trials investigating the pleiotropic effects of statins on endothelial dysfunction are sparse. Therefore, this present study aimed to evaluate the effects of statins on endothelial function beyond the effects on cholesterol reduction. The combination of the cholesterol-absorption inhibitor ezetimibe with low dose atorvastatin served as a tool to study the effect of high dose versus low dose atorvastatin on endothelial function with comparable cholesterol reduction in both groups. Based on the assumption of functional importance of pleiotropic effects of statins we hypothesized that therapy of patients with CAD with atorvastatin 80 mg

* Corresponding author at: II. Medizinische Klinik, Klinikum der Johannes Gutenberg-Universität Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany.
Tel.: +49 6131 17 3746; fax: +49 6131 17 6428.
E-mail address: warnholt@uni-mainz.de (A. Warnholtz).

0021-9150/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2008.11.032

per day (A80) causes improvement of endothelial dysfunction to a greater extent than 10 mg atorvastatin + 10 mg ezetimibe per day (A10E10). In order to test this hypothesis, endothelial function of the forearm conductance vasculature of patients with CAD was determined by ultrasonic measurement of flow-mediated vasodilation of the brachial artery, an accepted method to evaluate endothelial function of peripheral conductance arteries.

1. Methods

1.1. Patient sample

Fifty-eight patients with CAD (defined as at least one coronary stenosis >50% or general wall irregularities), an LDL-cholesterol of >100 mg/dl and endothelial dysfunction of the brachial artery (defined as flow-mediated dilation <6%) were included in the study. The most relevant exclusion criteria were the presence of an acute coronary syndrome, pre-treatment with ezetimibe, statins, fibrates or colestipol within the previous 3 months, initiation of ACE inhibitor-, AT1-receptorblocker- or calcium channel blocker therapy within the previous 4 weeks, serum creatinine >2.0 mg/dl, elevated liver enzymes >1.5 times the upper normal limit, elevated creatine kinase >3 times upper normal limit or overt heart failure with an left ventricular ejection fraction of <30%. Written informed consent was obtained. The study protocol was approved by the independent ethics committees of the chamber of physicians of Hamburg and of Rhineland Palatinate, Mainz, Germany. Investigations were undertaken in accordance to the Declaration of Helsinki. The study was registered as a randomized controlled trial titled "Effect of coadministration of ezetimibe with statin therapy versus statin therapy alone on flow-mediated vasodilation in patients with coronary artery disease" (CEZAR) with the ISRCTN34110682 - http://www.controlled-trials.com/ISRCTN34110682.

1.2. Study protocol

Patients were randomized to take oral atorvastatin 80 mg or atorvastatin 10 mg + ezetimibe 10 mg per day for 8 weeks. In a double-blind fashion all patients took two identically looking capsules per day containing atorvastatin 40 mg each or atorvastatin 10 mg and ezetimibe 10 mg, respectively. The study course consisted of three visits. At visit 1 the baseline ultrasound study was performed and blood as well as urine samples were collected. Visit 2 was held after 2 weeks for collection of blood samples for CK, ALT, AST, serum creatinine in order to detect side effects of the study drugs. After 8 weeks at visit 3 vascular function was reassessed by ultrasound studies. Blood and urine samples were collected for biochemical analysis.

1.3. Assessment of vascular function

Endothelium-dependent, flow-mediated dilation (FMD) and nitroglycerin-induced endothelium-independent dilation (NMD) of the right brachial artery were non-invasively examined by two-dimensional high resolution ultrasonic imaging as described previously [4]. Image acquisition and analysis were performed in a blinded manner. Briefly, two-dimensional images of the right brachial artery and pulsed-Doppler flow velocity signals were obtained with a 12 MHz linear array transducer on an HDI5000 ultrasound system (Philips Medical Systems, Hamburg, Germany). Imaging was performed in a dark quiet room at a temperature of 21–23 °C. Long-acting nitrates and calcium channel blockers were withheld for more than 16 h before each measurement. Patients rested in the supine position for at least 10 min before the first scan and remained supine until the final recording was acquired. Images were obtained approximately 5 cm above the antecubital crease. First, baseline two-dimensional images were acquired followed by pulsed-Doppler blood flow velocity with the signal at a 67° angle to the vessel lumen and the 1.0 mm wide gate positioned at the center of the artery. In order to induce hyperemia, a 3.5″ wide blood pressure cuff was inflated at the upper arm to 50 mmHg above systolic blood pressure or at least 200 mmHg. Arterial occlusion was kept for 5 min with the transducer carefully maintained in the identical position. The cuff was then rapidly deflated and pulse-Doppler velocity signals were recorded for 5 s. At 60 s after cuff deflation two-dimensional images of the brachial artery were recorded for a period of 5 s. After 15 min of recovery a second baseline measurement was taken. In order to examine the nitroglycerin-induced endothelium-independent dilation of the brachial artery, sublingual nitroglycerin (0.8 mg) was administered and brachial artery measurements were obtained after 4 min as described above. Brachial artery diameters were analyzed in a 10 mm segment before and after induction of reactive hyperemia and upon application of nitroglycerin, respectively. Special care was taken to analyze identical segments by the identification of anatomical landmarks. The brachial artery segment diameter was determined by commercially available edge detection software (Brachial Analyzer, Medical Imaging Application, Iowa City, USA). The diameter resulted from the average of five end-diastolic frames. Flow-mediated dilation was calculated as the percent change in brachial artery diameter in response to hyperemia. Brachial artery blood flow at rest and during reactive hyperemia was determined by the average of the flow velocity time integral of the first three beats multiplied by the vessel cross-sectional area and the heart rate. The relative increase in blood flow during reactive hyperemia was expressed as the percent increase in flow from baseline.

1.4. Biochemical analyses

A venous blood sample was obtained after overnight fast. Plasma cholesterol, HDL, triglycerides, fasting glucose and uric acid were measured using an automated analyzer. LDL-cholesterol was calculated by the Friedewald formula. Plasma C-reactive protein (CRP) was measured using a high-sensitive immunoturbidimetric method (Tina-quant CRP, Roche Diagnostics). Urinary concentrations of 8-iso-prostaglandin F2alpha as marker for oxidative stress were determined in spot samples by gas chromatography–tandem mass spectrometry as described previously [5] and were corrected by urinary creatinine concentration to account for differences in renal excretory function. Large batches containing samples from visits 1 and 3 of the same patients were analyzed in order to exclude the influence of day-to-day variation of the assay.

1.5. Statistical analysis

The primary endpoint variable of the study was the absolute change of brachial artery flow-mediated dilation before and after treatment in the A80 compared to the A10E10 group. Based on previously published studies our study was designed to have 80% power to demonstrate a difference of 2.0 percent points of FMD change between the treatment groups, with a sample size of 29 patients per group. Data were analyzed in the intention-to-treat population that was defined by the existence of two valid FMD measurements (baseline and follow-up). Comparison of changes in variables between the groups was calculated using the unpaired Student's *t*-test or Mann–Whitney rank sum test when normality testing failed. Comparison of the variables within the groups before and after treatment was calculated using the paired Student's *t*-test (SPSS15.0, SPSS Inc.). Correlations between changes in FMD and LDL-cholesterol concentration were tested by calculation of Pearson's correlation coefficient. Clinical characteristics of the two study groups were compared using the unpaired Student's *t*-test or chi-