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# Rosuvastatin improves pulse wave reflection by restoring endothelial function

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ABSTRACT

One of the major indicators of intact endothelial function is basal nitric oxide (NO) activity. Further, it seems to be likely that statin therapy exerts beneficial effects on vascular function, at least in part via an improvement of NO bioavailability. In the present double-blind crossover study 29 hypercholesterolemic patients were randomly assigned to receive rosuvastatin and placebo for 42 days. Pulse wave analysis was assessed after 30 min of rest (baseline) and after infusion of $N^G$-monomethyl-L-arginine (L-NMMA) at the end of 42 days treatment period. The magnitude of the increase in central augmentation index (cAIx) in response to inhibition of NO synthase (NOS) by L-NMMA is indicative of basal NO activity. CAIx was significantly lower (18.3 ± 10 versus 21.9 ± 12%, p = 0.027) with rosuvastatin compared to placebo. There was no increment of cAIx in response to L-NMMA in placebo group. In contrast, cAIx increased significantly in response to L-NMMA (20.5 ± 11 versus 25.7 ± 10 mm Hg, p = 0.001) in rosuvastatin group. The percentage of increase of cAIx tended to be more pronounced after treatment with rosuvastatin compared to placebo (53.7 ± 92 versus 14.1 ± 36%, p = 0.087). Pulse pressure amplification (PPA) improved (1.31 ± 0.2 versus 1.26 ± 0.2%, p = 0.016) after rosuvastatin compared to placebo. Regression analyses revealed that both LDL-cholesterol and CRP-levels are independent determinants of basal NO activity improvement, which itself is an independent determinant of vascular function, expressed by an improvement of pulse wave reflection and PPA. In this placebo controlled study, treatment with rosuvastatin improved vascular and endothelial function. Determinants for improved NO production in patients with hypercholesterolemia were the achieved levels of LDL-cholesterol and CRP. Overall, in patients without CV disease, rosuvastatin exerted beneficially effect on vascular dysfunction, one of the earliest manifestation of atherosclerosis.



## Introduction

Atherosclerosis is a dynamic process which begins to affect arteries early in life and the endothelium contributes to its initiation, perpetuation and clinical manifestations (Ross, 1999; Vanhoutte, 2009). The endothelium plays a key role in the regulation of vascular tone and regional blood flow via production and release of a variety of vasoactive substances (Vane et al., 1990). One of the most important mediators released by the endothelium is nitric oxide (NO), which is generated by a variety of nitric oxide synthases (NOS), including endothelial NOS (eNOS) (Govers and Rabelink, 2001). Endothelium-derived NO has been widely used as clinical marker of endothelial function (Wolfrum et al., 2003). Endothelial dysfunction (ED), defined as alterations in the normal properties of the endothelium that are inappropriate for preservation of organ function, is characterised by loss of protective endothelial characteristics in favour of deleterious mechanisms. It is among others characterised by altered production and/or decreased bioavailability of NO (Stehouwer, 2004), which can be directly assessed by systemic infusion of $N^G$-monomethyl-L-arginine (L-NMMA) in humans. It was shown that endothelial dysfunction or rather decreased NO bioavailability is one of the earliest manifestations of atherosclerosis, before the appearance of structural changes in the vasculature (Bugiardini et al., 2004; Halcox et al., 2002; Werns et al., 1989). ED is evident in nearly all risk factors for cardiovascular disease (CVD), like hypertension (John and Schmieder, 2000), diabetes (Calver et al., 1992), smoking (Celermajer et al., 1993) and hypercholesterolemia (Chowienczyk et al., 1992).

Interestingly, mentioned CV risk factors (e.g. hypercholesterolemia) are also associated with augmentation index (AIx) (Brooks et al., 1999; Mahmud and Feely, 2003; Wilkinson et al., 2002b) and AIx independently predict both the premature coronary artery disease (CAD) and an increased risk for severe short and long-term cardiovascular (CV) events in patients undergoing pecutaneous coronary intervention (Weber et al., 2005; 2004). In past sometimes AIx was simplistically considered to be an index of arterial stiffness.

*Abbreviations:* (c)AIx, (central) augmentation index; BP, blood pressure; CAD, coronary artery disease; CV(D), cardiovascular (disease); (e)NO(S), (endothelial) nitric oxide (synthase); GFR, glomerular filtration rate; L-NMMA, $N^G$-monomethyl-L-arginine; PP(A), pulse pressure (amplification); PWV, pulse wave velocity.

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However, AIx is a composite measure of the magnitude of wave reflection and the timing of wave reflection, which itself is affected by an earlier return of the pulse wave as a result of an increased pulse wave velocity (PWV) due to arterial stiffness. The dissociation of AIx and arterial siffness, expressed as PWV, is strengthened by findings that AIx has been shown to predict CV and all-cause mortality even in end-stage renal disease patients with normal PWV (London et al., 2001), thus AIx is a direct measure of pulse wave reflection.

Furthermore, the endothelium is proposed to be an important regulator of pulse wave reflection. This is based on the observation that competitive NOS blockade with L-NMMA increase AIx (pulse wave reflection) in healthy patients (McVeigh et al., 2001; Wilkinson et al., 2002a). Moreover, this association is strengthened by findings of several studies showing that AIx (pulse wave reflection) is directly correlated with endothelial dysfunction in coronary as well as forearm vascular beds in hypertensive patients and controls (Ceravolo et al., 2003; Ichigi et al., 2005; McEniery et al., 2006).

There is no doubt that statin treatment improves endothelial function also in patients with hypercholesterolemia (Egashira et al., 1994; John et al., 1998; Perticone et al., 2000). Interestingly, although data are conflicting there is accumulating evidence that the therapeutic approach of statin therapy also has beneficial effects on pulse wave reflection. Recently, two studies have shown that statin treatment improves pulse wave reflection, expressed as significant regression of augmentation index normalised to a heart rate of 75 beats per minute (AIx@75) (Efrati et al., 2007; Forst et al., 2008).

Therefore, we examined in the current study the effect of rosuvastatin therapy on both pulse wave reflection and basal NO activity representing endothelial function, determined by assessing the increase of cAIx@75 during competitive NOS blockade with L-NMMA (Wilkinson et al., 2002a), in hypercholesterolemic patients.

## Methods

### *Study cohort*

This investigator initiated trial followed a double-blind, randomised, placebo-controlled cross-over design in 29 subjects with hypercholesterolemia. Subjects were recruited by advertisement in a local newspaper and were enrolled consecutively. Written informed consent was obtained from each patient prior to study inclusion. Inclusion criteria were age between 18 and 75 years, fasting LDL-cholesterol level of $\geq$160 and <250 mg/dl, fasting triglycerides <350 mg/dl while not receiving lipid-lowering drugs for at least 4 weeks prior to commencement of the study. Main exclusion criteria included diabetes, micro- or macroalbuminuria, smoker, history of serious hypersensitivity reaction to statins, or statin induced myopathy, homozygous familial hypercholesterolemia, hyperlipoproteinemia type III and any renal, hepatic or cardiovascular disease. All had an estimated glomerular filtration rate (GFR) of >60 ml/min.

The study protocol was approved by the local ethics committee and the study was performed according to Declaration of Helsinki and "good clinical practice" (GCP) guidelines. The study was registered at www.clincaltrials.gov (ID: NCT00160745).

The sponsor AstraZeneca did not contribute to data collection, interpretation of the data, or the decision to approve and submit the manuscript.

### *Study design*

Patients who fulfilled all inclusion criteria were randomly assigned to receive study medication according to our study design. Treatment consisted of identically appearing and tasting capsules (kindly provided by AstraZeneca) containing either rosuvastatin 10 mg or placebo. They were advised to take 1 tablet of study medication (rosuvastatin or placebo) each morning for 42 days. At day 42, pulse wave analysis was performed. After a 2-week washout phase, the second course of randomised study medication started and pulse wave analysis was performed after again 42 days of treatment.

### *Pulse wave analysis (measurement of pulse wave reflection)*

All subjects were studied in supine position in a quiet and temperature-controlled room. Brachial BP was measured with an oscillometric device (Dinamap Pro 100 V2; Criticon, Norderstedt, Germany) and averages of the last three measurements were taken. Immediately thereafter, radial artery waveforms were sampled in the same arm by non-invasive technique, calibrated to the brachial BP, with the commercially available SphygmoCor™ System (AtCor Medical, Sydney, Australia). In brief, radial artery waveforms were recorded from the radial artery at the wrist, using high-fidelity applanation tonometer (Millar Instruments, Houston, Tex.), directly into the SphygmoCor™ System. Radial artery waveform was averaged from single waveforms recorded consecutively for 8 s. Corresponding central (aortic) waveforms were then automatically generated from the radial artery waveform by a validated transfer function (Karamanoglu et al., 1993; Pauca et al., 2001; Wilkinson et al., 1998). From the derived central waveforms, data are given for central systolic, diastolic BP and AIx defined as the difference between the second and the first systolic peaks, expressed as percentage of central pulse pressure (PP). The central augmentation index (cAIx) was also normalised to a heart rate of 75 beats per minute (cAIx@75). Therefore, different baseline levels of heart rate or changes in heart rate due to provocative manoeuvres, like e.g. L-NMMA, did not contribute to changes in AIx. All recordings included in the analysis had high-quality, defined as in-device quality index >80% (as derived from an algorithm that includes average pulse height, pulse height variations, diastolic variations and the maximum rate of rise of the peripheral waveform). PP amplification is determined as the ratio of peripheral PP to central PP.

### *Assessment of basal NO activity*

Basal NO activity in the arterial system was assessed by determining the change of cAIx@75 during administration of the competitive NOS inhibitor L-NMMA (Clinalfa, Läufelingen, Switzerland) (Wilkinson et al., 2002a). After 115–120 min of rest, pulse wave analysis was done (as described above), representing baseline levels. Thereafter, L-NMMA was administered intravenously as a bolus infusion (3 mg/kg body weight) over 5 min, followed by constant infusion with a rate of 0.05 mg/kg/min for 5 min. During the last 3 min of infusion, measurements of pulse wave analysis were repeated. Basal NO activity was defined as change of cAIx@75 between the measurements during L-NMMA administration and baseline. For safety reasons, an infusion of 100 mg/kg body weight of L-arginine (L-arginine hydrochloride 6%, University Hospital Pharmacy, Erlangen, Germany) was infused over 30 min to counteract L-NMMA induced vasoconstriction.

### *Statistical analyses*

Data of clinical characteristics are expressed as mean $\pm$ SD. Paired and unpaired Student *t* tests were used for comparisons where appropriate. Where indicated, a multiple stepwise linear regression analysis with significance levels of 0.05 for entry and 0.10 for removal of a variable at each forward step was conducted. Differences were considered to be statistically significant, if the two-sided P-value was <0.05. The change of cAIx in response to L-NMMA between rosuvastatin and placebo group is given in percentage change, thereby taking potential differences in cAIx at baseline into account. All analyses were performed using the SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).