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Rosuvastatin improves basal nitric oxide activity of the renal vasculature in patients with hypercholesterolemia

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Abstract

Objective: Impaired endothelium-dependent vasodilation represents an early manifestation of atherosclerosis. Prospective studies have demonstrated that impaired endothelial function in the peripheral circulation of hypercholesterolemic patients predicts CV events and can be restored by statin treatment. Whether this also holds true in the renal circulation has not yet been adequately addressed.
Methods: In a double-blind, randomized, placebo-controlled cross-over trial, 40 hypercholesterolemic patients were randomly assigned to receive rosuvastatin (10 mg/day) and matching placebo. The primary objective of the study was to assess the effect of 6-week treatment with rosuvastatin on basal NOS activity of the renal vasculature, as assessed by measuring renal plasma flow (RPF) both before and after blockade of NOS with systemic infusion of N^G-monomethyl-L-arginine (L-NMMA). In a subgroup of 20 patients we also studied the effects of a 3-day treatment regimen.
Results: Compared to placebo treatment, rosuvastatin decreased LDL-cholesterol levels both after 3 days and 6 weeks of treatment. The decrease in RPF in response to L-NMMA was significantly more pronounced after 6-week therapy with rosuvastatin compared to placebo ($-13.7 \pm 1.0\%$ versus $-11.3 \pm 0.7\%$; $p = 0.046$), indicating increased basal NOS activity with rosuvastatin treatment. A trend towards improved basal NOS activity was already evident after 3-day treatment.
Conclusion: Treatment with rosuvastatin improved basal NOS activity in the renal circulation of hypercholesterolemic patients, suggestive of a nephroprotective effect. In view of the close relation between altered renal function and cardiovascular events, these nephroprotective effects may contribute to the improved CV prognosis associated with statin treatment.

Keywords: Hypercholesterolemia; Endothelium; Nitric oxide (NO); L-NMMA; Rosuvastatin

1. Introduction

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 [1]. Nitric oxide (NO) represents an important endothelium derived relaxing factor which is generated by a variety of nitric oxide synthases (NOS), including endothelium derived NOS (eNOS) [2]. Hypercholesterolemia has clearly been demonstrated to impair endothelium-dependent vasodilation and several mechanisms have been proposed to contribute to hypercholesterolemia-induced endothelial dysfunction. For example, oxidized-LDL-cholesterol (ox-LDL) has been shown to down-regulate eNOS expression, to decrease NO production and release, and to inactivate nitric oxide through scavenging by superoxide anion production [3]. Accordingly, interventional studies suggested that the beneficial effects of statins appear to be mediated by counteracting these mech-

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anisms [4,5] and provided clear evidence for a reduction of cardiovascular events with statin treatment in patients with hypercholesterolemia [6,7] and an up-regulation of eNOS expression was evident in response to statin treatment [8]. Furthermore, there is growing evidence to suggest that the beneficial effects of statin treatment are not solely mediated by their LDL-cholesterol lowering effect but also by additional pleiotropic effects [9,10]. However, the effects of statins appear to be diverse and depend upon the vasculature studied [9,10].

Despite numerous studies assessing endothelium-dependent vasodilation in the peripheral and coronary circulation under pathological conditions, no investigations have been carried out so far to assess whether *basal* NOS activity, as assessed by infusion of the NOS inhibitor L-NMMA, is altered in the renal circulation in patients with hypercholesterolemia. Similarly, the effect of statin treatment on *basal* NOS activity of the renal vasculature has not yet been investigated. This issue is of crucial importance, particularly in view of the available evidence suggesting that impaired renal function and increased albumin excretion, both of which are critically linked to renal endothelial function, predict cardiovascular prognosis in these patients [11–13]. Of note, Hillege et al. suggested a continuous relation between albuminuria and cardiovascular (CV) risk, even at levels of albumin excretion within the normal range [11], implying that urinary albumin leakage may reflect generalized integrity of the systemic vasculature and indicates early alterations of endothelial function. Accordingly, preservation or restoration of endothelial function of the renal vasculature emerges as a new and attractive therapeutic target in cardiovascular medicine [14]. Thus, in the current study we aimed to test the hypothesis that treatment with rosuvastatin improves NOS activity in the renal circulation of hypercholesterolemic patients when compared to placebo treatment.

2. Methods

2.1. Study design

In a double-blind, randomized, placebo-controlled cross-over trial volunteers from the area of Erlangen-Nürnberg, Bavaria, Germany, were randomly assigned treatment with identically appearing and tasting capsules (provided by AstraZeneca) containing either rosuvastatin 10 mg or placebo. After a thorough description of the study and a physical examination written consent was obtained from each patient prior to study inclusion. Inclusion criteria were age between 18 years and 75 years, fasting LDL-cholesterol level of $\geq$160 mg/dl 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. Exclusion criteria included history of serious hypersensitivity reaction to statins, or statin induced myopathy, diabetes mellitus (HbA1c > 6.1%), micro- or macroalbuminuria, uncontrolled arterial hypertension (systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg), any cardiovascular or cerebrovascular event within the last 12 weeks, severe cardiac pathologies, homozygous familial hypercholesterolemia, hyperlipoproteinemia type III and liver or kidney disease. The study protocol was approved by the local ethics committee (University of Erlangen-Nürnberg, Germany) and the study was performed according to "good clinical practice" (GCP) guidelines. Patients who fulfilled all inclusion criteria were randomly assigned to receive study medication according to our study design (Fig. 1).

2.2. Objectives

The primary objective of this study was to evaluate the effect of rosuvastatin compared to placebo on basal NOS activity of the renal circulation after 42 days of treatment ($n = 40$).

A secondary objective, that was additionally assessed in a subgroup ($n = 20$) of the participating patients was to determine the effect of rosuvastatin compared to placebo on basal NOS activity after short-term therapy for only 3 days. The rationale for these additional measurements were previous reports demonstrating improvements of vascular endothelial function already after short-term therapy with statins. The role of potential confounding factors known to influence endothelial function such as LDL-cholesterol, asymmetric dimethylarginine (ADMA), and age was also assessed.

2.3. Clearance protocol

All clearances were performed at the same time in the morning in a quiet and temperature-controlled room. To determine renal plasma flow (RPF) and glomerular filtration rate (GFR), the constant infusion input clearance technique was employed, as suggested by Cole et al. [15]. Two intravenous lines were placed, one for infusion of *p*-aminohippurate sodium (PAH), inulin, and N^G-monomethyl-L-arginine (L-NMMA), and one for blood sampling. After a priming dose of PAH (Nephrotest®, Merck) and inulin (Inutest®, Fresenius, Graz, Austria) that was adjusted according to body weight, a constant infusion of both tracer substances was given to achieve steady-state conditions. After reaching steady state conditions (115–120 min) duplicate blood samples were collected for assessment of baseline RPF and GFR, and the infusion of PAH and inulin continued.

To assess basal NOS activity of the renal circulation, RPF was determined both before and after infusion of the NOS inhibitor L-NMMA. Typically, infusion of L-NMMA results in a decrease of RPF through blockade of NO synthesis. The magnitude of the decrease in RPF in response to inhibition of NOS by L-NMMA depends on the prevailing activity, i.e. the higher basal NOS activity, the more pronounced is the decrease in RPF in response to L-NMMA and vice versa. A