Original Article

Effect of simvastatin on renal function in autosomal dominant polycystic kidney disease

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Abstract

Background. In animal models, HMG-CoA reductase inhibitors were able to improve renal function and endothelium-dependent vascular reactivity. In various experimental renal diseases, including autosomal dominant polycystic kidney disease (ADPKD), HMG-CoA reductase inhibitors improved the rate of decline in renal function. We studied the effect of simvastatin on ADPKD patients.

Methods. In a double-blind cross-over study, 10 normocholesterolaemic ADPKD patients were treated in random order for 4 weeks with 40 mg simvastatin or placebo daily. After each treatment period, we investigated the effect of simvastatin on renal blood flow and endothelium-dependent vascular reactivity. These periods were separated by a 4-week wash-out period.

Results. After treatment with simvastatin, glomerular filtration rate (GFR) significantly increased from 124±4 ml/min to 132±6 ml/min (P<0.05). Simultaneously, effective renal plasma flow (ERPF) increased significantly from 494±30 ml/min to 619±67 ml/min after simvastatin treatment (P<0.05). These renal effects were accompanied by a significantly enhanced vasodilator response to acetylcholine in the forearm after simvastatin treatment. Total serum cholesterol levels were significantly reduced after treatment with simvastatin, from 4.24±0.32 to 3.17±0.22 mmol/l (P<0.001).

Conclusion. We concluded that simvastatin treatment can ameliorate renal function in ADPKD patients, by increasing renal plasma flow, possibly via improvement of endothelial function. Long-term clinical trials with HMG-CoA reductase inhibitors are needed to confirm these results and to establish a chronic inhibiting effect of HMG-CoA reductase inhibitors on the progression towards end-stage renal disease in ADPKD patients.

Keywords: acetylcholine; autosomal dominant polycystic kidney disease; renal blood flow; renal function; simvastatin; vascular reactivity

Introduction

The progression of autosomal dominant polycystic kidney disease (ADPKD) towards end-stage renal disease (ESRD) is accompanied by glomerulosclerosis, vascular sclerosis, interstitial fibrosis, increased apoptosis of tubular cells and changes in the extracellular matrix [1–3]. Clinically, hypertension is known to be a risk factor for deterioration of renal function [4,5]. Activation of both the renin-angiotensin system (RAS) and the endothelial system probably plays a role in the pathogenesis of hypertension [6–9]. Various interventions are tested to influence one or more of the factors mentioned above in order to ameliorate the prognosis of ADPKD, such as treatment with ACE-inhibitors. The new class of cholesterol-lowering drugs, the HMG-CoA reductase inhibitors, may be another candidate for reducing decline in renal function in ADPKD patients. Experimental studies have shown that HMG-CoA reductase inhibitors improve the prognosis of various experimental renal diseases, including ADPKD [10–13]. This improvement is accompanied by a direct haemodynamic effect, resulting in an increase of renal blood flow, consequently followed by increase of glomerular filtration rate (GFR) [10–12,14,15]. Treatment with HMG-CoA reductase inhibitors also seems to prevent the development of hypertension in spontaneously hypertensive rats [16], and improve endothelium-dependent vascular reactivity [17,18]. These effects seem to be relatively independent of the cholesterol lowering effects of the...
HMG-CoA reductase inhibitors, but seem to depend on the interference of these drugs with the mevalonate pathway [17,18].

The mevalonate pathway is not only important for cholesterol synthesis but also for cell-signalling processes [19]. HMG-CoA reductase is essential for conversion of HMG-CoA to mevalonate. Inhibition of this pathway by an HMG-CoA reductase inhibitor leads to anti-proliferative effects, and changes in endothelial function. The anti-proliferative effects can lead to improvement of the prognosis of renal disease by reducing mesangial proliferation, monocyte invasion, and extracellular matrix accumulation, and increasing apoptosis in the proximal tubule [20–23]. Improvement of endothelial function is probably responsible for changes in vascular reactivity and is mediated by the ability of HMG-CoA reductase inhibitors to activate nitric oxide synthase [24,25]. The amelioration of vascular reactivity is likely to be responsible for the increase in renal blood flow seen in animal studies. The amelioration of vascular reactivity is a direct, short-term effect, in contrast to the benefit of the anti-proliferative effects of HMG-CoA reductase inhibitors, which are only likely to occur with chronic treatment.

The first objective of this study was to investigate whether the HMG-CoA reductase inhibitor simvastatin, in analogy to animal studies, is able to improve renal function by increasing RBF in ADPKD patients with normal to moderately elevated cholesterol levels. Furthermore, we studied the effect of simvastatin on endothelium-dependent vasodilatation in the forearm of these patients.

Patients and methods

Patients

A group of 10 untreated ADPKD patients (six males) was investigated, with normal to moderately elevated total cholesterol levels (3–6 mmol/l). Medical history, physical examination and routine laboratory tests did not reveal any abnormalities. All patients had a Cockroft clearance of > 50 ml/min. Patients were on a normal (approximately 180 mmol daily) sodium diet and none of them received medication. Three patients were smokers.

Study design

The study had a double-blind, randomized, placebo-controlled cross-over design. After a placebo run-in phase of 2 weeks, patients were treated in random order for 4 weeks with either 40 mg simvastatin once daily followed by 4 weeks treatment with placebo, or with placebo followed by 4 weeks treatment with 40 mg simvastatin. In both cases the two treatment periods were separated by a 4-week wash-out period. Every 2 weeks, cholesterol level, liver function and renal function were monitored. At the end of each treatment period the effect of simvastatin or placebo on renal haemodynamics and vascular reactivity was established. For ovulating women, each study day was planned in the same period of the menstrual cycles. At the study days, patients attended our clinical research unit at 8.00 am having fasted for 8 h and abstaining from alcohol, caffeine and nicotine for 12 h. The Medical Ethics Committee of the Leiden University Medical Center approved the protocol of the study, and informed consent was obtained. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Study protocol

The experiments were performed in a quiet room at a constant temperature of 22–24°C. After measuring forearm and hand volumes by water displacement, patients were placed in the supine position, with the non-dominant arm stabilized slightly above the level of the heart. After local anaesthesia of the skin, a 20-gauge polyethylene catheter (Ohmeda, Swindon, UK) was inserted in the brachial artery of the non-dominant arm for determination of blood pressure and infusion of vasoactive drugs. A venous cannula was inserted in the antecubital vein of the contra lateral arm for infusion of inulin and para-amino hippurate (PAH) to measure renal parameters. Heart rate was recorded from a triple lead electrocardiogram. The subjects rested for 60 min after the insertion of the intra-arterial catheter to achieve a stable baseline.

After the resting period of 60 min, renal parameters were studied by measuring GFR and effective renal plasma flow (ERPF). Inulin (Fresenius, Graz, Austria) and PAH were infused using Harvard volumetric precision pumps (Harvard ‘22’, Harvard Apparatus Ltd, Edenbridge, Kent, UK). First, a loading dose of inulin/PAH was given in 10 min, followed by continuous infusion of 4 h or more, dependent on the duration of the haemodynamic measurements. To determine inulin/PAH concentrations, four blood samples were drawn from the arterial cannula in the non-dominant arm after each infusion experiment, starting at least 90 min after the loading dose, when a steady state was reached. If arterial cannulation was not possible, blood samples were taken from an intravenous cannula in the non-dominant arm.

Five minutes after the loading dose of inulin/PAH, forearm vascular reactivity was measured by intra-arterial cumulative dose infusions of the endothelium-dependent vasodilator acetylcholine (1, 10, 100 and 1000 ng/kg/min) and the nitric oxide donor sodium nitroprusside (0.1, 1, 10 and 100 ng/kg/min) using Harvard volumetric precision pumps. Each single dose was given for 4 min. Finally, the infusion of acetylcholine was repeated together with a continuous infusion of the competitive nitric oxide synthase inhibitor 1-mono-methyl arginine (L-NMMA) in a dose of 30 μg/kg/min. The infusion of L-NMMA started 20 min prior to the acetylcholine infusion. The infusions of acetylcholine and sodium nitroprusside were given in random order, and a wash-out period of 30 min was allowed between the various infusions to allow forearm blood flow to return to basal levels. Forearm blood flow was measured using computerized R-wave triggered venous occlusion plethysmography as described previously [26,27]. Forearm blood flow was measured four times per minute and the averages of the last six measurements at the end of each dose step, when a steady state had been reached, were used for further analysis. During the measurements of forearm blood flow, the hand was excluded from the circulation, using a small wrist cuff inflated to 40 mmHg above the systolic blood pressure.
Drugs and solutions

Simvastatin was obtained from Merck, Sharp and Dohme (Haarlem, The Netherlands) and the matching placebo was prepared by the pharmacy of the Leiden University Medical Center. All active substances were prepared on the day of the study as sterile pharmaceutical solutions in the pharmacy of the Leiden University Medical Center. Acetylcholine (OPG, Utrecht, The Netherlands) and L-NMMA (Brunschurg, Amsterdam, The Netherlands) were dissolved in 0.9% saline. Sodium nitroprusside (E. Merck, Darmstadt, Germany) was dissolved in 5% dextrose.

Biochemical analysis

Plasma creatinine, liver function parameters, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, inulin, and PAH were measured using standard laboratory methods.

Statistical analysis

GFR was calculated for each patient by dividing the amount of inulin infused per minute by the plasma inulin level at blood sampling. ERPF was calculated, based on PAH infusion rate and levels, in the same manner. Average values of forearm blood flow and intra-arterial pressure were obtained from the last six recordings of each dose step, and used for calculation and construction of dose-response curves. The effects of simvastatin therapy were analyzed by Student’s paired two-tailed t-test for renal parameters and repeated-measures ANOVA for dose-response effects in the human forearm. Results are expressed as means (SE); a P-value of less than 0.05 was considered statistically significant.

Results

From the 10 ADPKD patients recruited, renal parameter data were available from nine, because venous cannulation failed in one male subject. Forearm blood flow data were available from six patients (5 males), because in four patients arterial cannulation did not succeed. The clinical characteristics of the patients are shown in Table 1. Compared to placebo treatment, total serum cholesterol was significantly reduced after the 4 weeks treatment period with 40 mg simvastatin from 4.24 ± 0.32 mmol/l to 3.17 ± 0.22 mmol/l (P < 0.001) (Table 2). No side-effects of simvastatin treatment were noted. Simvastatin had no effect on serum creatinine kinase levels.

Table 1. Clinical characteristics of ADPKD patients (n = 10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>Gender (males/females)</td>
<td>6/4</td>
<td>6/4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 ± 0.03</td>
<td>1.77 ± 0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 ± 3</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135 ± 4</td>
<td>135 ± 4</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>90 ± 3</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>92 ± 9</td>
<td>92 ± 9</td>
</tr>
</tbody>
</table>

Table 2. Plasma lipid levels during placebo and after treatment with 40 mg simvastatin daily

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>Placebo</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.24 ± 0.32a</td>
<td>3.17 ± 0.22</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.01 ± 0.20</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.15 ± 0.27</td>
<td>1.69 ± 0.21</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.56 ± 0.41</td>
<td>1.13 ± 0.23</td>
</tr>
</tbody>
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*P < 0.001.

Fig. 1. Effect of simvastatin treatment on glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). After 4 weeks of treatment with simvastatin, GFR and ERPF increased in all but one patient. Analysis with paired t-test showed significance (P < 0.05 for both GFR and ERPF).
Glomerular filtration fraction (FF) decreased from 25% to 23% during treatment with simvastatin (NS, data not shown).

Effects of simvastatin on forearm blood flow

The intra-arterial infusions of acetylcholine, sodium nitroprusside and l-NMMA did not influence blood flow in the contralateral arm, heart rate and blood pressure, excluding important systemic haemodynamic effects and effects on renal vasculature. Changes in forearm blood flow may, therefore, be interpreted as local vascular effects of the drugs in the forearm. Compared to placebo, basal forearm blood flow (2.48 ± 0.59 ml/100 ml/min) was not significantly influenced by simvastatin treatment (2.52 ± 0.61 ml/100 ml/min). Intra-arterial blood pressure was not affected by treatment with simvastatin.

During placebo treatment, both the cumulative dose infusions of acetylcholine and sodium nitroprusside (Figure 2) induced significant dose dependent increase in forearm blood flow. The concomitant infusion of l-NMMA significantly attenuated the acetylcholine induced vasodilatation (P < 0.05) (data not shown).

After simvastatin treatment, the acetylcholine-induced forearm vasodilatation was significantly enhanced compared to the response during placebo treatment (P < 0.05) (Figure 2), whereas sodium nitroprusside elicited a similar vascular response during placebo and simvastatin treatment (Figure 2).

Discussion

In this study we describe two beneficial effects of a HMG-CoA reductase inhibitor in ADPKD patients. First, this study shows a favourable effect of 4 weeks of 40 mg simvastatin daily on both GFR and ERPF in ADPKD patients that has never been reported before. These findings corroborate experimental data, showing a positive effect of HMG-CoA reductase inhibitors on renal function in various animal models of chronic renal failure [12,14,15]. In these studies it was demonstrated that the increase in renal function was accompanied by an increase in renal blood flow. The increase in renal blood flow, after treatment with an HMG-CoA reductase inhibitor, was achieved by decrease of efferent renal vessel diameter and increase of afferent vessel diameters [12,29]. These pregglomerular and postglomerular changes would increase the single-nephron glomerular filtration rate and therefore explain the observed increase in GFR. However, in our study, there was a trend towards a decrease in FF after treatment with simvastatin. An explanation for this phenomenon would be speculative since we did not measure the effect of simvastatin on afferent and efferent vessel diameters. However, this suggests a larger vasodilative effect on the afferent vessels than a vasoconstrictive effect on the efferent vessels. The decrease in FF may also contribute to an ameliorative effect on progression of renal function loss as was demonstrated for angiotensin-converting enzyme inhibitors in diabetic patients [30].

The favourable effects on GFR and ERPF/RBF in our study were demonstrated in only a small group of patients. However, all patients except one showed
amelioration of renal function. In such a small group, significance could be due to inaccuracy of the used method. We used the constant infusion technique of insulin and PAH without urine collection, which has been shown to be an accurate and reproducible method [28]. In a recent study, the effect of menopause, gender and oestrogen replacement therapy on vascular NO activity was described [31]. Data on renal function were extracted from a patient group that showed equal distribution of gender (5 males, 4 females). The females were all premenopausal, had a regular menstrual cycle or used oral contraceptives. Although we did not measure FSH or oestrogen levels, caution was taken to plan the study days in the same period of their menstrual cycle.

The second finding of this study comprises the enhanced endothelium-dependent responsiveness in ADPKD patients after simvastatin treatment. In the present study it was shown that the acetylcholine-induced vasodilatation in the forearm of these patients, was significantly enhanced after 4 weeks of treatment with 40 mg simvastatin. That the acetylcholine-elicited vasodilatation was mediated by nitric oxide was confirmed by the fact that this response was attenuated by the nitric oxide synthase inhibitor L-NMMA. This ameliorative effect of simvastatin on endothelial function is confirmed by an earlier study on hypercholesterolaemic humans [17]. Our data on forearm blood flow were conceived predominantly in males (1 female). Because male gender is associated with reduced arterial NO activity [31], it may be suggested that the simvastatin-induced amelioration of endothelium function in this study is merely due to a gender effect. However, the study by Stroes et al. [17] was performed with predominantly females (3 males, 5 females, all premenopausal). In a rat model of ADPKD, and more lately also in ADPKD patients, impaired endothelium-dependent relaxation of resistance vessels was demonstrated [9,32]. Altogether, these findings suggest that statins are able to restore impaired endothelial function in hypercholesterolaemic patients, ADPKD patients, and possibly also males.

HMG-CoA reductase inhibitors repress the conversion of HMG-CoA to mevalonate, the metabolites of which are critical not only in growth and proliferation of cells, but also in signalling post-transcriptional mechanisms, as was demonstrated by the upregulation of endothelial NO synthase by a HMG-CoA reductase inhibitor [25]. The findings in our study that simvastatin was able to improve RBF and vascular reactivity is probably explained by this direct effect of statins on NO production. Moreover, the suggestion has been made that in ADPKD patients NO synthase is impaired [32]. The pre-existent endothelial dysfunction in ADPKD patients, may explain the effect of simvastatin in absence of hypercholesterolemia. In ADPKD patients, dysfunction of the RAS has been suggested several times as a cause of hypertension [6,7]. The shear stress of hypertension accompanied by the supposed higher tissue levels of angiotensin II should lead to higher expression of NO synthase and, therefore, NO. Impairment of NO synthase in this situation could deteriorate hypertension and its detrimental effect on renal function. Interestingly, the four patients with the highest blood pressure, showed the least increase (and the least adverse effect) in GFR and ERPF after simvastatin treatment. It may be hypothesized that in hypertensive, or in our case borderline hypertensive ADPKD patients, further upregulation of NO synthase was impaired by longstanding hypertension and angiotensin II production. It seems unlikely that the effect of statins on mesangial proliferation or extracellular matrix accumulation are responsible for the amelioration of renal function in our patients. However, with chronic treatment these anti-proliferative effects of HMG-CoA reductase inhibition may become apparent.

In conclusion, the present study suggests that simvastatin treatment can ameliorate renal function in normotensive ADPKD patients, by increasing renal plasma flow possibly via improvement of endothelial function. Although these results are promising, caution must be taken because the study group was very small and heterogeneous. Long-term clinical trials with HMG-CoA reductase inhibitors are needed to confirm these results and to establish a possible chronic inhibiting effect of HMG-CoA reductase inhibitors on the progression towards end-stage renal disease in ADPKD patients.

References

Renal and vascular effects of simvastatin in ADPKD


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