Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model

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Zafar I, Tao Y, Falk S, McFann K, Schrier RW, Edelstein CL. Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model. Am J Physiol Renal Physiol 293: F854–F859, 2007. First published June 20, 2007; doi:10.1152/ajprenal.00059.2007.—Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening hereditary disease and is the fourth most common cause of end-stage kidney disease. Preclinical studies to identify effective interventions to prevent or slow progression of PKD nephropathy are therefore direly needed. Heterozygous Han:SPRD rats are an autosomal dominant PKD model with many of the characteristics of AD-PKD in humans. In the present study, parameters known to antedate the decrease in renal function, namely, renal structure, renal blood flow (RBF), and mean arterial pressure (MAP), were evaluated with three different interventions, namely, HMG-CoA reductase inhibition with lovastatin, angiotensin-converting enzyme (ACE) inhibition with enalapril, and a combination of these two treatments. The statin therapy demonstrated structural and functional benefits, including increased RBF and decreased BUN, independently of a change in MAP, while the ACE inhibition therapy demonstrated structural benefit in association with a decrease in MAP. An enhancement of these protective interventions in this autosomal dominant PKD model was not demonstrated with the combined treatment.

renal blood flow; renal structure; blood pressure

A DECREASE IN RENAL BLOOD flow (RBF) by magnetic resonance imaging recently has been shown in patients with ADPKD to parallel the increase in total kidney volume and to precede the decline in glomerular filtration rate (GFR) (27). In a double-blind crossover study in 10 normocholesterolemic ADPKD patients, treatment for 4 wk with 40 mg simvastatin vs. placebo demonstrated an increase in RBF and GFR (30). Other HMG-CoA reductase inhibitors (i.e., statins) have been shown in animal models to ameliorate progression of nephropathy and to increase RBF (1.12). The statin, cerivastatin, decreased blood pressure, serum creatinine, and albuminuria in rats transgenic for human renin and angiotensin with hypertension, cardiac hypertrophy, renal damage, and marked albuminuria (20).

There is considerable evidence that the renin-angiotensin system (RAS) is activated in ADPKD patients. The plasma renin and aldosterone responses to posture and angiotensin-converting enzyme (ACE) inhibition were greater in ADPKD patients than patients with essential hypertension of comparable age, gender, blood pressure, renal function, and urinary sodium excretion (3). Angiotensinogen, ACE, angiotensin II type I (AT1) receptor, and angiotensin II have been found within cysts and tubules of ADPKD kidneys, thus implicating intrarenal RAS (19). Immunoreactive renin also has been found in tubules and cyst fluid of ADPKD kidneys (26).

The effect of statin therapy with lovastatin to diminish the severity of renal disease in an autosomal dominant model of polycystic kidney disease, male Han:SPRD rats, has been reported (11). The effect of statin therapy on mean arterial pressure (MAP) and RBF in this model has, however, not been reported. It is also not known whether the effect of statin therapy occurs by modulation of the RAS or by an independent pathway. The present study, therefore, has focused on the effect of HMG-CoA reductase inhibition with lovastatin on MAP, RBF, GFR, kidney volume, and cyst density in the Han:SPRD rats compared with ACE inhibition with enalapril. The combination of statin and ACE inhibition on renal function and structure was also examined.

METHODS

Animals. The study was conducted in heterozygous (Cy+/+) and normal littermate control (+/+H1) Han:SPRD rats. All the normal rats and Cy+/+ rats studied were males. The Cy+/+ Han:SPRD rat develops clinically detectable polycystic kidney disease by 8 wk of age, as evidenced by a doubling of kidney size and kidney failure compared with +/+ control rats (4, 22). A colony of Han:SPRD rats was established in our animal care facility from a litter that was obtained from the Polycystic Kidney Program at the University of Kansas Medical Center. The study protocol was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. Rats had free access to tap water and standard rat chow.

Experimental protocol. Male Cy+/+ and +/+ rats were weaned at 3 wk of age and then divided into three groups. Group 1 was treated with either lovastatin (4 mg·kg−1·day−1·sc) or vehicle (100% ethanol). Group 2 was treated with enalapril (50 mg/l) in the drinking water or drinking water that did not contain enalapril. Water intake was monitored. The mean water intake for each rat for the 5-wk duration of the study was 817 ± 19 ml. Thus the mean dose of enalapril per rat for the 5-wk period was 40.8 mg. Group 3 was treated with lovastatin (4 mg·kg−1·day−1·sc) and enalapril (50 mg/l) in the drinking water and the vehicle for both lovastatin and enalapril. Treatments were for 5 wk from 3 to 8 wk of age. In all the studies, littermates were used as controls.Lovastatin was obtained from Merck Sharp & Dohme Research Laboratories (Rahway, NJ). The dose of lovastatin was based on rat studies in ADPKD (11), experimental nephrotic syndrome (13), and tissue selectivity studies in rats in vivo. Enalapril was obtained from Sigma (St. Louis, MO). The dose of enalapril used has a significant antihypertensive effect in rats 3–12

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wk of age (15, 18). At the end of the 8 wk of age, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt) and subjected to blood pressure and RBF studies before the kidneys were removed and weighed. The left kidney was fixed in 4% paraformaldehyde in PBS for 120 min and then put into 70% ethanol and embedded in paraffin for histological examinations.

Cyst volume density. Hematoxylin-eosin-stained sections were used to determine the cyst volume density. This was performed by a reviewer, blinded to the identity of the treatment modality, using point-counting stereology (5). Areas of the cortex at 90, 180, and 270° from the hilum of each section were selected to guard against field selection variation.

Chemistry. Blood urea nitrogen (BUN) levels were measured using a Beckman autoanalyzer (Beckman Instruments, Fullerton, CA).

Blood pressure and RBF. Blood pressure was measured by cannulation of the femoral artery (31). RBF was measured with a Transonic flow probe placed around the main renal artery (31). Briefly, the animals were anesthetized with pentobarbital sodium and placed on a thermostatically controlled surgical table. A tracheotomy was performed, at which time a steady steam of 100% oxygen was blown over the tracheal tube throughout the experiment. Catheters (custom pulled from PE-250) were placed in the jugular vein for maintenance infusion and the carotid artery for blood pressure determinations. The kidney was exposed by a left subcostal incision and was dissected free from perirenal tissue, and renal arteries were isolated for the determination of RBF using a blood flowmeter and probe (0.5v; Transonic Systems, Ithaca, NY) as described by Traynor and Schnermann (29). MAP was measured via a femoral artery catheter connected to a Transpac IV transducer and monitored continuously using Windaq Waveform recording software (Dataq Instruments).

Statistical analysis. Data were analyzed in two ways. 1) In three individual experiments a one-way ANOVA was used with a posttest according to Newman-Keuls (see Figs. 1–4); PKD plus vehicle for lovastatin, PKD plus lovastatin, and normal littermate controls; PKD plus vehicle for enalapril, PKD plus enalapril, and normal littermate controls; and PKD plus vehicles for both lovastatin and enalapril, PKD plus the combination of lovastatin and enalapril, and normal littermate controls. 2) Experiments were combined to create a 2 × 2 factorial design. After the data from the three separate experiments from the PKD rats only (lovastatin vs. vehicle, enalapril vs. vehicle, and lovastatin+enalapril vs. vehicle) were combined, a two-way ANOVA was performed with lovastatin at two levels: “yes” or “no” and enalapril at two levels, yes or no. The Tukey-Kramer P value adjustment was used for post hoc multiple comparisons.

In both analyses, a P value of < 0.05 was considered statistically significant. Values are expressed as means ± SE.

RESULTS

Lovastatin therapy in PKD. Treatment of the PKD rats from 3 to 8 wk of age with lovastatin (4 mg·kg⁻¹·day⁻¹ sc) vs. vehicle was associated with significant differences (Fig. 1). The percent increase in weight of both kidneys corrected for body weight was significantly less in the lovastatin-treated animals compared with the vehicle-treated animals. Similarly, lovastatin treatment decreased the percentage of cyst volume density compared with the vehicle-treated PKD animals. This beneficial effect of lovastatin on cystic kidneys was only partial, since the kidney weights and cyst volume density percentages in the lovastatin-treated PKD animals were still greater than those same parameters in the littermate controls.

The effect of lovastatin on kidney structure was accompanied by a significant increase in RBF compared with vehicle-treated PKD rats. These hemodynamic and structural benefits were associated with a significant decrease in BUN in the lovastatin-treated PKD rats. The structural, functional, and RBF results occurred in the absence of differences in MAP (Fig. 2A) and body weight (data not shown).

Enalapril therapy in PKD. ACE inhibition in the PKD rats from 3 to 8 wk of age with enalapril was associated with a decrease in percentage of kidney weight per body weight and cyst volume density (Fig. 3). In contrast to lovastatin treatment, these differences in kidney structure with enalapril treatment were not associated with significant differences in BUN and RBF. There was, however, a significant decrease in MAP in the
enalapril-treated PKD animals compared with the untreated PKD rats and littermates (Fig. 2B). There were no differences in body weight (data not shown).

**Lovastatin and enalapril therapy in PKD.** Since the structural changes in PKD of lovastatin and enalapril were associated with different effects on RBF and MAP, the combination of these two therapeutic approaches was examined (Fig. 4). The combination therapy significantly decreased the percentage of kidney weight per body weight and cyst volume density. The significant decrease in BUN persisted with the combined therapy, but a significant increase in RBF was not observed. MAP was significantly decreased with the combined therapy (Fig. 2C). Body weight was significantly lower in the combined vs. vehicle treatment group (282 ± 12 vs. 223 ± 9 g, P < 0.01).

**Two-way ANOVA.** A two-way ANOVA was performed with lovastatin at two levels: yes or no and enalapril at two levels, yes or no. The following four groups were analyzed: 1) PKD rats (Cy+/−), no treatment; 2) PKD rats, enalapril; 3) PKD rats, lovastatin; and 4) PKD rats, enalapril and lovastatin.

There was a significant main effect of lovastatin (P = 0.0334) and a main effect of enalapril (P = 0.0041) on the percentage of kidney weight per body weight, but no interaction effect (P = 0.1093). There was a near-significant main effect of lovastatin (P = 0.0611), but no main effect of enalapril (P = 0.2100) on body weight, and no interaction effect (P = 0.1571). There was no main effect of lovastatin

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Fig. 2. Mean arterial pressure (MAP; mmHg). Lovastain therapy did not have a significant effect on MAP (A). Angiotensin-converting enzyme inhibitor (ACEI; enalapril) therapy (B) and a combination of ACEI and lovastatin therapy (C) significantly reduced MAP in PKD rats.

Fig. 3. Enalapril therapy in PKD. The ACEI enalapril significantly reduced 2K/TBW(%) and cyst volume density in PKD rats. Enalapril did not have a significant effect on BUN and RBF (n = 4−8/group).
on cyst volume density. After an adjustment for multiple comparisons, treatment with lovastatin (18.7 ± 0.5, \( P = 0.0040 \)), enalapril (18.7 ± 2.0, \( P = 0.0039 \)), or lovastatin+enalapril (21.1 ± 5.5, \( P = 0.0220 \)) lowered cyst volume density compared with no treatment (30.7 ± 1.1). Thus there was no synergistic effect of the combination of lovastatin and enalapril on cyst volume density.

**DISCUSSION**

ADPKD is the most common life-threatening hereditary disease affecting from 1–400 in 1,000 Americans (8). ADPKD is more common than cystic fibrosis, sickle cell disease, Huntington’s chorea, Down’s syndrome, and myotonic dystrophy combined. Preclinical studies to detect potential interventions that will slow the progression of ADPKD to end-stage renal disease (ESRD) are, therefore, very important.

ADPKD is the fourth most common cause of ESRD in this country. There are no perfect animal models of ADPKD; however, the heterozygous Han:SPRD rat model is widely used and has several features which mimic ADPKD in humans (22). This animal model has autosomal dominant inheritance, progresses slowly to ESRD with hypertension and uremia, exhibits interstitial fibrosis and inflammation, and is more aggressive in male animals.

The progression of renal disease and hypertension in ADPKD in both children and adults is associated with increases in kidney volume and cystic volume density (10, 19, 25). These structural changes were, therefore, examined in the present study. The HMG-CoA reductase inhibitor lovastatin had been previously used in this autosomal dominant male PKD model because of its antiproliferative and anti-inflammatory effects which occur independently of effects on cholesterol. The current results confirm the effect of lovastatin to significantly decrease the kidney weight, cyst volume density, and BUN after early treatment from 3 to 8 wk after birth. Early treatment is relevant, since ADPKD is clinically apparent in children as evidenced by increased renal volume, blood pressure, and left ventricular mass index (8).

Since progression of ADPKD nephropathy correlates not only with renal structural and functional changes but also with blood pressure and RBF, the effects of these hemodynamic parameters were examined in the present study. There was no effect of lovastatin on MAP despite the above beneficial results. Of interest, however, was the effect of lovastatin to significantly increase RBF in this autosomal PKD model. As with most preventive interventions, intervention in ADPKD to slow or prevent progression of nephropathy may best occur before a decrease in GFR or rise in BUN. Increased kidney and cyst volumes by magnetic resonance imaging (2), an increase in RBF by magnetic resonance angiography (27), and hypertension (7, 17, 23) have emerged in ADPKD as early predictors of progression, before a decrease in GFR. Thus monitoring renal structure changes, MAP, and RBF in preclinical intervention trials is important. The current study is the first to report the effects of statin therapy on MAP and RBF on this model of ADPKD.

Since ADPKD is a variety of benign neoplasia, combination therapies may be necessary to aggressively thwart renal progression. In this regard, combination therapy with lisinopril and simvastatin was used in a rat model of passive Heymann

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**Fig. 4.** Combination lovastatin and enalapril therapy in PKD. Combination therapy with the ACEI enalapril and lovastatin significantly reduced 2K/TBW(%), cyst volume density, and BUN in PKD rats. The combination therapy significantly increased RBF (n = 3–6/group).
nephritis (34). In this study, combination therapy significantly limited glomerulosclerosis, tubular damage, and interstitial inflammation compared with vehicle or the individual drugs alone. As noted below, the combination of enalapril and lovastatin therapy in the present study did not have an enhanced effect on PKD compared with the individual drugs alone.

The RAS, along with sodium retention, has been shown to be involved in the early hypertension in ADPKD (3, 28, 32). Moreover, inhibition of the RAS with ACE inhibition has been shown in prospective, randomized studies to better reverse left ventricular hypertrophy (24) and decreased urinary albumin excretion (6) in ADPKD patients than calcium channel blockers at comparable effects on blood pressure. A meta-analysis has shown to have a beneficial effect of ACE inhibition on renal progression in patients with baseline protein excretion rates of 1 g/day or greater (14). Studies of RAS inhibition on renal progression in the Han:SPRD rat, however, are conflicting. The ACE inhibitor enalapril and angiotensin receptor blocker losartan were shown to decrease kidney weight, histology score, and serum creatinine compared with hydralazine treatment in Han:SPRD rats (15). However, the MAP values were much lower in the enalapril and losartan than hydralazine treatment groups. Another study using early treatment with the ACE inhibitor cilazapril demonstrated a significant decrease in blood pressure, serum creatinine, and renal cyst volume in this autosomal model of PKD (21). In contrast, however, another study was unable to observe an effect on renal progression, as assessed by serum urea concentration, in Han:SPRD rats with enalapril treatment (33). The ACE inhibition treatment was, however, started later in these studies, and surprisingly no effect of enalapril on blood pressure was observed. In the current study, the same dose of enalapril (50 mg/l drinking water) was shown to significantly decrease MAP whether administered alone or with lovastatin. In the present study, MAP was measured by direct arterial cannulation vs. tail plethysmography in the previous study (33). ACE inhibition in the current study demonstrated a significantly decreased kidney weight and cyst volume density but not BUN, RBF was not increased with enalapril treatment. Thus, in the ADPKD model the functional benefit, as assessed by BUN, was better with statin than ACE inhibition treatment.

The effects of the two agents on PKD structure were comparable. The effects of lovastatin to decrease kidney weight, cyst volume density, BUN, and to increase RRF were sustained when combined with enalapril. The effect of enalapril to decrease MAP was sustained when combined with lovastatin, but combined therapy did not enhance the effect on renal structure. Currently, an National Institutes of Health–funded, prospective, randomized study comparing ACE inhibition vs. ACE inhibition plus statin therapy in ADPKD children on renal function, albuminuria, endothelial function, and left ventricular mass index has been initiated.

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