The Effect of Ruboxistaurin on Nephropathy in Type 2 Diabetes

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OBJECTIVE — Ruboxistaurin selectively inhibits protein kinase C-β and ameliorates kidney disease in animal models of diabetes. The purpose of this study was to evaluate the effects of ruboxistaurin on diabetic nephropathy in humans.

RESEARCH DESIGN AND METHODS — A randomized, double-blind, placebo-controlled, multicenter, pilot study was performed to evaluate the effects of 32 mg/day ruboxistaurin for 1 year in persons ($n = 123$) with type 2 diabetes and persistent albuminuria (albumin-to-creatinine ratio [ACR] 200-2,000 mg/g), despite therapy with renin-angiotensin system inhibitors. The primary end point was a change in the ACR. Estimated glomerular filtration rate (eGFR) (four-component equation from the Modification of Diet in Renal Disease study) was also calculated.

RESULTS — At baseline, urinary ACR was 764 ± 427 mg/g (means ± SD), and eGFR was 70 ± 24 ml/min per 1.73 m^2. Systolic and diastolic blood pressures were 135 ± 14 and 75 ± 9 mmHg, respectively. HbA_{1c} was 8.0 ± 1.2%. After 1 year, urinary ACR decreased significantly (−24 ± 9%) in participants treated with ruboxistaurin ($P = 0.020$) and nonsignificantly (−9 ± 11%) in the placebo group ($P = 0.430$). The ACR-lowering effect of ruboxistaurin appeared by 1 month. eGFR did not decline significantly in the ruboxistaurin group (−2.5 ± 1.9 ml/min per 1.73 m^2) ($P = 0.185$), whereas the placebo group lost significant eGFR over 1 year (−4.8 ± 1.8 ml/min per 1.73 m^2) ($P = 0.009$). Between-group differences for changes in ACR and eGFR were not statistically significant, but this pilot study was underpowered to determine such differences.

CONCLUSIONS — In persons with type 2 diabetes and nephropathy, treatment with ruboxistaurin reduced albuminuria and maintained eGFR over 1 year. Ruboxistaurin may add benefit to established therapies for diabetic nephropathy.

Diabetes Care 28:2686–2690, 2005

Nephropathy develops in ~40% of people with type 2 diabetes and is the leading cause of end-stage renal disease in the U.S. and the developed world (1,2). Established therapies include antihypertensive treatment (particularly with ACE inhibitors or angiotensin receptor blockers [ARBs]), glycemic control, and limitation of dietary protein (3–9). Many persons with diabetes have progressive nephropathy even with these therapies. Therefore, treatments targeting novel pathogenic mechanisms may be beneficial. Preclinical studies have shown an important role for protein kinase C (PKC)-β in the pathogenesis of diabetic nephropathy. This signal transduction mediator induces a number of processes leading to kidney injury that can be prevented by ruboxistaurin, a selective PKC-β inhibitor, in diabetic animals (10,11). The effect of ruboxistaurin on diabetic nephropathy in humans has not been previously evaluated. The purpose of this study was to determine whether treatment with ruboxistaurin in persons with type 2 diabetes and persistent albuminuria, despite therapy with renin-angiotensin system inhibitors, would improve indicators of nephropathy after 1 year.

RESEARCH DESIGN AND METHODS

— Participants had type 2 diabetes, were ≥30 years old, and received stable doses of an ACE inhibitor, ARB, or both for at least 6 months before screening. Doses of ACE inhibitors and ARBs were recommended based on those approved for treatment of hypertension. Participants were required to have albuminuria, defined as an albumin-to-creatinine ratio (ACR) between 200 and 2,000 mg/g in two of three urine samples (collected up to 1 week apart) with values that did not vary by >20%. Women and men were excluded for serum creatinine levels >1.7 or >2.0 mg/dl, respectively. Other major exclusion criteria included kidney transplant, mean arterial blood pressure >110 mmHg, HbA_{1c} (A1C) >11%, congestive heart failure, abnormal liver function tests, cancer within 5 years, concomitant use of strong inhibitors of cytochrome P450 3A4, and initiation of lipid-lowering therapy within 3 months of screening. The study was conducted at 17 clinical sites in the U.S. (see APPENDIX). Institutional review boards at each site approved the study. Participants provided written, informed consent before entering the study. The ethical principles of the Declaration of Helsinki and guidelines on good clinical practice were strictly followed.

Participants underwent a complete evaluation, including blood chemistry, hematologic assessment, and urinary ACR tests. Those who met selection criteria were randomly assigned to treatment with either 32 mg/day ruboxistaurin or placebo for 1 year. This ruboxistaurin dose produces clinically relevant effects in persons with diabetic retinopathy and neuropathy (P.W.A., unpublished obser-

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Received for publication 18 February 2005 and accepted in revised form 14 June 2005.

K.R.T. and R.D.T. serve on advisory panels and are paid consultants for Eli Lilly. G.L.B. has been a paid consultant for Eli Lilly. J.B.M. served on an advisory panel and as a paid consultant for and has received honoraria from Eli Lilly.

Abbreviations: ARB, angiotensin receptor blocker; ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; PKC, protein kinase C.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying editorial, p. 2803.

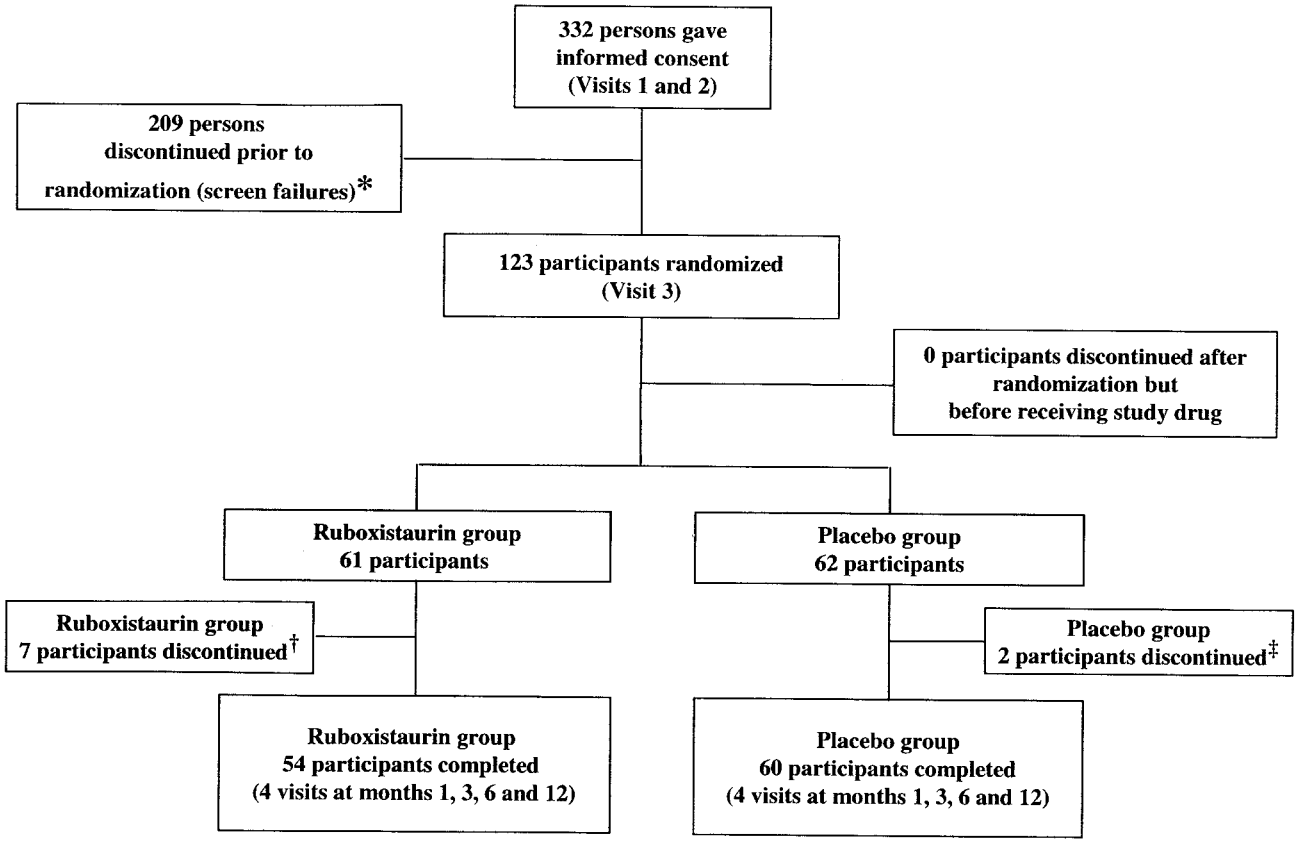

Figure 1—*Disposition of persons who gave informed consent and participants who were randomized. *Reasons for screen failure: ACR too low* (n = *146), serum creatinine too high* (n = *17), ACR too high* (n = *15), ACR too variable* (n = *9), A1C too high* (n = *8), mean arterial blood pressure >110 mmHg* (n = *1), or miscellaneous other* (n = *13). †Reasons for discontinuation in the ruboxistaurin group: metastatic cancer of unknown primary origin* (n = *1), libido decreased* (n = *1), mental status changes* (n = *1), or personal conflict* (n = *4). ‡Reasons for discontinuation in the placebo group: personal conflict* (n = *2).*

vations). Randomization was stratified by mean arterial blood pressure (80–90, 91–97, 98–103, and 104–110 mmHg). A centralized randomization system generated the concealed allocation sequence. Investigators used a telephonic interactive voice system to obtain treatment assignment. The interactive voice system confirmed correct assignment by requiring entry of a confirmation code. Participants, investigators, study coordinators, and the sponsor were blinded to treatment assignment. Evaluations were performed at baseline and at months 1, 3, 6, and 12. At each follow-up visit, urine samples for ACR, blood pressure, and adverse events were evaluated. At 6 and 12 months, complete evaluations were repeated. ACE inhibitors and/or ARBs were continued throughout the study.

Urinary ACR and estimated glomerular filtration rate

The primary end point was a reduction in urinary ACR. Urinary albumin was assayed on a Behring nephelometer II using polyclonal antisera to purified human albumin (intra-assay coefficient of variation [CV] = 4.3%; interassay CV = 4.4%). Creatinine was measured on a Roche Hitachi analyzer using a modified Jaffe reaction (intra-assay CV = 0.8–1.6%; interassay CV = 1.7–2.2%). Testing was performed at a central laboratory (Covance, Indianapolis, IN). Estimated glomerular filtration rate (eGFR) was calculated using the four-component equation from the Modification of Diet in Renal Disease study: eGFR (ml/min per 1.73 m^2) = $186 \times S_{Cr}^{-1.154} \times$ (age [years])$^{-0.203} \times$ (0.742 if female) or $\times$ (1.210 if African American).

Statistics

Because this was a pilot trial of a novel treatment, an a priori power analysis was not performed. The investigators who designed the trial (K.R.T., G.L.B., R.D.T., and P.W.A.) planned to enroll 50 participants per treatment group. Allowing for a drop-out rate up to 15%, the total enrollment goal was set at 120. The primary outcome was prespecified as a change from baseline to end point in log-ACR and analyzed by ANCOVA with factors for treatment assignment, baseline log-ACR, and investigative site. The natural logarithm transformation was used for ACR values because of the highly skewed nature of the data. A superiority test was performed to determine whether ruboxistaurin treatment would produce a ≥20% (with 95% CIs) reduction in ACR compared with placebo after 1 year. Within-group changes were also analyzed in this model, with one-sample *t* tests used to evaluate differences from baseline in each treatment group. For eGFR, a similar ANCOVA analysis was used, without logarithmic transformation. Dichotomous variables were analyzed using Fisher's exact test, and other continuous variables were analyzed using ANOVA with a factor for treatment assignment. Comparisons were made on an intent-to-treat basis. Analyses of the change from baseline necessarily included only patients with both baseline and follow-up

Table 1—*Baseline characteristics of participants*

	Placebo	RBX	All	*P* value
n	62	61	123	
Age (years)	62 ± 9	61 ± 10	62 ± 10	0.673
Men	41 (66)	47 (77)	88 (72)	0.231
Caucasian	45 (73)	40 (66)	85 (69)	
African American	15 (24)	13 (21)	28 (23)	0.341
Other race	2 (3)	8 (13)	10 (8)	
Current smokers	12 (19)	21 (34)	33 (27)	0.069
Alcohol use	13 (21)	17 (28)	30 (24)	0.407
Urinary ACR (mg/g)	800 ± 436	728 ± 424	764 ± 427	0.353
eGFR (ml/min per 1.73m^2)	69 ± 24	71 ± 26	70 ± 24	0.912
BMI (kg/m^2)	36 ± 6	35 ± 8	35 ± 7	0.246
Systolic blood pressure (mmHg)	136 ± 14	134 ± 15	135 ± 14	0.347
Diastolic blood pressure (mmHg)	76 ± 9	74 ± 10	75 ± 9	0.322
Mean arterial blood pressure (mmHg)	96 ± 9	94 ± 9	95 ± 9	0.159
A1C (%)	7.7 ± 1.1	8.2 ± 1.0	8.0 ± 1.2	0.052
Total cholesterol (mmol/l)	4.8 ± 1.0	4.7 ± 1.4	4.8 ± 1.2	0.193
LDL cholesterol (mmol/l)	2.6 ± 0.8	2.5 ± 0.9	2.5 ± 0.8	0.402
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.192
Triglycerides (mmol/l)	2.5 ± 1.4	3.1 ± 6.3	2.8 ± 4.5	0.374

Data are means ± SD or *n* (%). For race, the single *P* value refers to comparison between Caucasian, African American, and other. RBX, 32 mg/day ruboxistaurin.

values; the last-observation-carried-forward approach was applied to missing follow-up values. Data were analyzed with SAS, version 8.02, and presented as means ± SD, unless otherwise noted. Statistical significance was defined as $P < 0.05$.

RESULTS — Between June 2002 and May 2003, 123 of 332 persons who gave informed consent were randomized (Fig. 1). Baseline characteristics and medication use did not differ between treatment groups (Tables 1 and 2). Blood pressure and A1C did not change over the course of the study (Table 3). Although this study included men and women, as well as individuals of diverse ethnicity, it was too small to analyze effects by sex or race.

Albuminuria and renal function

At baseline, urinary ACR values were similar between groups: 728 ± 424 and 800 ± 436 mg/g in ruboxistaurin- and placebo-treated participants, respectively (Table 1). After 1 year, those who received ruboxistaurin experienced a significant −24 ± 9% decrease ($P = 0.020$) in ACR, whereas those receiving the placebo demonstrated a nonsignificant −9 ± 11% change ($P = 0.430$) (Table 3). The ACR-lowering effect of ruboxistaurin appeared as early as 1 month. The ACR reduction in ruboxistaurin-treated participants was not statistically different from that with the placebo, but this pilot study was not powered to determine such a difference.

Baseline eGFR was similar in the ruboxistaurin- and placebo-treated groups: 71 ± 26 and 69 ± 24 ml/min per 1.73 m^2, respectively (Table 1). Participants in the placebo group lost significant eGFR: −4.8 ± 1.8 ml/min per 1.73 m^2 ($P = 0.009$) over 1 year (Table 3). In contrast, loss of eGFR was not significant in ruboxistaurin-treated participants: −2.5 ± 1.9 ml/min per 1.73 m^2 ($P = 0.185$). Although changes in eGFR did not differ between groups, this analysis was also underpowered.

Safety

Serious adverse events occurred in 9 placebo-treated participants and 15 of those who received ruboxistaurin, including 2 deaths. One was attributed to metastatic cancer of unknown primary origin, and the other occurred after bilateral subdural hematomas due to a fall. The only statistically significant difference between groups with regard to treatment-emergent adverse events was that episodes of hypertension requiring intervention were not reported in participants receiving ruboxistaurin, whereas such episodes occurred in 8% of placebo-treated participants ($P = 0.006$).

CONCLUSIONS — This pilot study was conducted to assess the effect of ruboxistaurin on nephropathy in persons with type 2 diabetes and persistent albuminuria, despite receiving the current standard of care, including ACE inhibitors and/or ARBs. After 1 year, participants in the ruboxistaurin group had a reduction in urinary ACR, whereas those in the placebo group did not. The ACR-lowering effect of ruboxistaurin treatment occurred by 1 month. Renal function, examined by eGFR, was maintained with ruboxistaurin treatment. In contrast, participants in the placebo group had a significant decline in eGFR that was within the range reported in recent clinical trials of ARBs and ACE inhibitors in persons with type 2 diabetes and nephropathy (6,7,12). Ruboxistaurin may add benefit to these therapies, as reflected in decreased albuminuria and maintenance of renal function.

The rationale for this pilot study was based on a key role of PKC-β in the pathogenesis of diabetic kidney disease in animal models. PKC encompasses a group of at least 12 isoforms that are important signal transduction mediators leading to cellular growth, fibrosis, and tissue injury (13). In diabetes, the β isoforms are expressed and activated in vascular target organs (10,14). Ruboxistaurin displays a high degree of specificity for inhibiting PKC-β. In a rat model of type 1 diabetes induced by streptozotocin and in the *Lepr^{db}/Lepr^{db}* mouse model of type 2 diabetes, ruboxistaurin normalized glomerular hyperfiltration, reduced albuminuria, attenuated mesangial expansion, and/or prevented induction of profibrotic matrix proteins in glomeruli (10,11). Furthermore, in a severely hypertensive and diabetic model, a rat transgenic for renin exposed to streptozotocin, ruboxistaurin treatment reduced albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis (15).

This is the first clinical study performed to evaluate the effects of ruboxistaurin on diabetic nephropathy in

Table 2—*Medication use at baseline*

	Placebo	RBX	All	*P* value
n	62	61	123	
Insulin	48 (77)	44 (72)	92 (75)	0.539
Lipid-lowering drugs	43 (69)	38 (62)	81 (66)	0.451
Statin	43 (69)	36 (59)	79 (64)	0.262
Fibrate	7 (11)	4 (7)	11 (9)	0.530
ACE inhibitor	46 (74)	44 (72)	90 (73)	0.841
ARB	28 (45)	23 (38)	51 (42)	0.466
Both ACE inhibitor and ARB	12 (19)	6 (10)	18 (15)	0.202
Calcium channel blockers	33 (53)	26 (43)	59 (48)	0.281
Nondihydropyridine	10 (16)	4 (7)	14 (11)	0.154
Dihydropyridine	23 (37)	23 (38)	46 (37)	0.999
Diuretics	40 (65)	33 (54)	73 (59)	0.274
β-Blocker	26 (42)	21 (34)	47 (38)	0.459
Other antihypertensive agents	11 (18)	16 (26)	27 (22)	0.283
Antihypertensive agents taken per participant (*n*)	4.1 ± 1.5	3.6 ± 2.0	3.9 ± 1.8	0.052

Data are *n* (%) or means ± SD. RBX, 32 mg/day ruboxistaurin.

humans. Based on levels of albuminuria and eGFR, the participants typically had stage 2 chronic kidney disease, as defined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative staging system (16). They were treated according to current guidelines for diabetes and chronic kidney disease (17,18). This type of multiple risk factor intervention has been shown to reduce the risk of renal and cardiovascular complications in people with type 2 diabetes and nephropathy (19). The degree of glycemic control achieved was consistent with that found in other clinical trials in this population (6,7,12,19). Blood pressure was treated with an average of approximately four agents, including ACE inhibitors and/or ARBs. In studies of ARBs for persons with type 2 diabetes and nephropathy, the relative risk reduction for renal outcomes was ~20% (6,7). Recent secondary analyses from the RENAAL (Reduction in End Points in Noninsulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan) trial have emphasized the importance of lowering albuminuria as an indicator of reduced renal and cardiovascular risk (20,21). The additional benefits of ruboxistaurin to lower albuminuria and maintain renal function in diabetic persons already treated for multiple risk factors are noteworthy.

Table 3—*Change from baseline in urinary ACR and eGFR, blood pressure, and A1C at follow-up visits*

Treatment	*n*	1 month	3 months	6 months	12 months
Urinary ACR change (%)*					
Placebo	62	−16 ± 7†	−9 ± 8	−9 ± 10	−9 ± 11
RBX	59	−24 ± 7†	−28 ± 6†	−29 ± 8†	−24 ± 9†
Urinary ACR change (mg/g)‡					
Placebo	62	17 (424)	−37 (413)†	21 (661)	26 (896)†
RBX	59	−60 (363)†§	−139 (417)†§	−136 (598)	−121 (481)
eGFR change (ml/min per 1.73 m^2)					
Placebo	62	—	—	−2.7 ± 1.8	−4.8 ± 1.8†
RBX	57	—	—	−0.2 ± 1.9	−2.5 ± 1.9
Systolic blood pressure (mmHg)					
Placebo	62	135 ± 16	135 ± 15	136 ± 16	138 ± 19
RBX	59	135 ± 15	136 ± 15	134 ± 16	134 ± 18
Diastolic blood pressure (mmHg)					
Placebo	62	77 ± 12	76 ± 8	76 ± 10	76 ± 10
RBX	59	74 ± 10	74 ± 9	73 ± 11	74 ± 10
A1C (%)					
Placebo	62	—	—	7.7 ± 1.1	7.7 ± 1.2
RBX	56	—	—	8.0 ± 1.3	7.9 ± 1.3

Data are means ± SD unless otherwise indicated. *ACR change from baseline (%) was calculated from log-transformed values using the ANCOVA model with least-square means (prespecified primary outcome). †Change from baseline, $P < 0.05$. ‡ACR change from baseline (mg/g) without log transformation reported as median (interquartile range for the middle two quartiles). §Difference between groups, $P < 0.05$. RBX, 32 mg/day ruboxistaurin.

This pilot study has important limitations. First, it was underpowered to detect between-group differences in urinary ACR or eGFR. Nevertheless, these initial observations provide a rationale to proceed with a larger, adequately powered trial to detect differences between groups and clinical outcomes. Second, although treatment with ACE inhibitors and/or ARBs was required, the agents and doses were not standardized. However, their usage was consistent with recommendations for treatment of hypertension and representative of usual practice. Third, although participants were well matched, we cannot exclude the possibility that changes in lifestyle or medications other than the study drug could have influenced the results. Finally, the frequency of adverse events was not significantly increased in the ruboxistaurin group, but the small sample size and limited duration of follow-up precludes firm conclusions about safety.

In summary, ruboxistaurin had favorable effects on albuminuria and renal function in persons with type 2 diabetes and nephropathy. The albuminuria-lowering effect occurred early and was sustained long term. Those treated with ruboxistaurin did not experience a significant decline in renal function. Large-scale trials should be performed to confirm its effectiveness and safety. Ruboxistaurin is a promising novel treatment that may improve upon established therapies for diabetic nephropathy.

Acknowledgments— Eli Lilly and Company supported this study. R.D.T. acknowledges support from the National Institute of Diabetes and Digestive and Kidney Diseases (5K24DK0281802).

The authors gratefully acknowledge the study investigators and coordinators (APPENDIX) and participants who made this study possible. We thank Keri Kles, PhD, for editorial assistance, Katherine Case for study coordination, and Drs. Dwight McKinney and Louis Vignati for critical review of the manuscript.

APPENDIX

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