



Inhibition of PKC by Oral Administration of Ruboxistaurin Is Well Tolerated and Ameliorates Diabetes-Induced Retinal Hemodynamic Abnormalities in Patients

Citation

Aiello, L. P. 2006. Inhibition of PKC by Oral Administration of Ruboxistaurin Is Well Tolerated and Ameliorates Diabetes-Induced Retinal Hemodynamic Abnormalities in Patients. Investigative Ophthalmology & Visual Science 47, no. 1: 86–92. doi:10.1167/iovs.05-0757.

Published version

<https://doi.org/10.1167/iovs.05-0757>

Link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:33776267>

Terms of use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material (LAA), as set forth at

<https://harvardwiki.atlassian.net/wiki/external/NGY5NDE4ZjgzNTc5NDQzMGIzZWZhMGFIOWI2M2EwYTg>

Accessibility

<https://accessibility.huit.harvard.edu/digital-accessibility-policy>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#)

Inhibition of PKC β by Oral Administration of Ruboxistaurin Is Well Tolerated and Ameliorates Diabetes-Induced Retinal Hemodynamic Abnormalities in Patients

Lloyd Paul Aiello,^{1,2} Allen Clermont,¹ Vipin Arora,³ Matthew D. Davis,⁴ Matthew J. Sheetz,³ and Sven-Eric Bursell¹

PURPOSE. To assess ocular and systemic safety and pharmacodynamic effects of the oral PKC β selective inhibitor ruboxistaurin (RBX; LY333531) mesylate in patients with diabetes.

METHODS. This was a double-masked, placebo-controlled, parallel, randomized, single-center clinical study evaluating the effect of oral administration of RBX (8 mg twice a day, 16 mg per day, or 16 mg twice a day) or placebo for 28 days in patients with no or very mild diabetic retinopathy on mean retinal circulation time (RCT), retinal blood flow (RBF), treatment-emergent adverse events, and other safety parameters.

RESULTS. Twenty-nine persons aged 18 to 65 years with type 1 or 2 diabetes were evaluated. The only treatment-emergent adverse event with a statistically significant difference among treatment groups was abdominal pain, which was more common in placebo-treated subjects ($P = 0.049$). Statistically significant effects of RBX were observed on several hematologic and laboratory parameters, but values were within the normal reference range and none of the changes was deemed clinically meaningful. In patients receiving 16 mg RBX twice daily, the diabetes-induced increase in RCT was ameliorated, with a baseline-to-endpoint difference of -0.84 seconds ($P = 0.046$) relative to placebo. Increasing RBX dose was linearly associated with greater effect on RCT ($P = 0.03$). Similar results were observed with RBF.

CONCLUSIONS. RBX was well tolerated at doses up to 16 mg twice daily for 28 days in patients with diabetes. It ameliorated diabetes-induced RCT abnormalities. No serious safety problems were identified in this patient population. Compared with prior published data, these findings represent the first direct human evidence of both bioavailability of RBX to retinal vessels and amelioration of diabetes-induced retinal hemodynamic abnormalities by an oral PKC β inhibitor. (*Invest Ophthalmol Vis Sci.* 2006;47:86–92) DOI:10.1167/iovs.05-0757

The link between metabolic alterations in diabetes mellitus and the incidence of diabetic microvascular complications,

such as neuropathy, retinopathy, and nephropathy, has long been suspected.^{1–3} However, the molecular mechanisms underlying these pathologic processes are incompletely understood. Substantial data suggest that diabetes-induced activation of protein kinase C (PKC) is an important event in the genesis of these microvascular abnormalities.^{4–9}

Hyperglycemia increases de novo synthesis of diacylglycerol (DAG), a physiological activator of PKC. Diabetes activates multiple isoforms of PKC in animals. However, the PKC- β 2 isoform is preferentially activated in tissues that usually demonstrate diabetes-induced damage such as retina, nerve, kidney, aorta, and heart.^{4,6,10}

Activation of PKC- β is associated with basement membrane thickening, leukocyte vascular adhesion, vascular permeability, and prolonged retinal circulation time,^{6,9,11–13} all classic pathologic changes observed in diabetes. In addition, PKC- β activity is critical for vascular endothelial growth factor (VEGF) signaling¹⁴ and VEGF is believed to be the primary mediator of retinal vascular permeability in diabetic macular edema and intraocular neovascularization in proliferative diabetic retinopathy,^{15,16} as well as being involved in a variety of other ischemic retinal disorders.^{17,18} The PKC- β inhibitor ruboxistaurin (RBX; LY333531) mesylate and its single equipotent metabolite *N*-desmethyl-RBX^{19–21} have been shown to ameliorate diabetes-induced abnormalities in retinal circulation in the rat.²¹ In other animal models, RBX reduced intraocular neovascularization caused by retinal ischemia²² and suppressed VEGF-induced retinal vascular permeability.¹⁸ It also decreased leukocyte adhesion in the retinal microcirculation of diabetic rats.²³

In a recently published randomized, placebo-controlled, multicenter clinical trial evaluating the progression of diabetic retinopathy as the primary endpoint, treatment with RBX for 36 to 46 months reduced the relative risk of moderate visual loss by 63% ($P = 0.012$) even accounting for potential confounding variables and despite having no effect on progression of diabetic retinopathy.²⁴ This effect was primarily evident in eyes with definite diabetic macular edema at baseline, reducing the risk of moderate visual loss sustained over 6 months from 25% in placebo to 10% in the 32 mg/d RBX-treated group ($P = 0.017$).

Such findings suggest that an oral PKC- β inhibitor may be an effective novel therapeutic agent for concurrently suppressing diabetic microvascular complications in a variety of body systems, despite suboptimal glycemic control. The goal of the present study was to provide the initial evaluation of RBX safety in patients with diabetes during repeated administration and to provide evidence that oral administration of this compound could reach the retinal microvasculature and demonstrate a positive pharmacodynamic effect. Indeed, these data are the first to provide human evidence of amelioration of diabetes-induced retinal hemodynamic abnormalities by an oral PKC- β inhibitor.

From the ¹Beetham Eye Institute and Eye Research Section, Joslin Diabetes Center, Boston, Massachusetts; the ²Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts; ³Lilly Research Laboratories, Indianapolis, Indiana; and the ⁴Fundus Photograph Reading Center, University of Wisconsin, Madison, Wisconsin.

Supported by Eli Lilly.

Submitted for publication June 15, 2005; revised September 7, 2005; accepted November 30, 2005.

Disclosure: L.P. Aiello, Eli Lilly (R, F, C); A. Clermont, Eli Lilly (F); V. Arora, Eli Lilly (E); M.D. Davis, Eli Lilly (C); M.J. Sheetz, Eli Lilly (E); S.-E. Bursell, Eli Lilly (F)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Lloyd Paul Aiello, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215; lpaiello@joslin.harvard.edu.

METHODS

Subjects

Individuals were eligible for this study if they had type 1 or 2 diabetes mellitus, were 18 to 65 years of age, and had an Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity grade of $\leq 20^{25}$ in at least one eye (none to very mild nonproliferative diabetic retinopathy), as determined by grading of seven ETDRS standard field stereoscopic color fundus photographs at the University of Wisconsin Fundus Photograph Reading Center. Subjects had to be free of severe or chronically disabling conditions other than diabetes and have an $HbA_{1c} \leq 11.0\%$ at baseline. Subjects were excluded if, in their otherwise eligible eye, they had clinically significant macular edema, a history of intraocular surgery, laser photocoagulation, or a vitreous hemorrhage. In addition, patients could not have an allergy to fluorescein dye, uncontrolled diabetes mellitus, impaired renal function (creatinine >2.0 mg/dL), anemia (hematocrit $<35\%$ or Hb <12 g/dL), impaired hepatic function (more than twice the upper normal limit for aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, alkaline phosphatase, and/or total bilirubin), or any other clinically significant abnormal laboratory value. Pregnant or lactating women or those of childbearing potential who were not practicing birth control were excluded. Subjects could not have participated in any other investigational trial within the 12 weeks before their baseline visit.

Study Design

In this double-masked, placebo-controlled, parallel, randomized, single-center study, the subjects were assigned to one of four groups after a 2- to 3-week screening and entry phase. The treatment groups were (1) 8 mg RBX twice daily ($n = 7$); (2) 16 mg RBX per day ($n = 7$); (3) 16 mg RBX twice daily ($n = 8$); or (4) placebo ($n = 7$). RBX-placebo was provided so that all subjects took two identical-appearing capsules twice daily. RBX was taken orally with a meal to maximize absorption. The dose regimen lasted 28 days for all treatment groups.

During the screening and entry period of the study, patients were evaluated using the tests listed below to determine eligibility. On the day of treatment group assignment (randomization visit), subjects received the first dose of study medication and stayed at the clinic for the remainder of the day for safety assessments. Subjects returned weekly for further safety and pharmacodynamic assessments until the 28-day follow-up visit was completed.

The following systemic safety assessments were performed before randomization and at each subsequent visit: collection of adverse events, pulse, respiration, systolic blood pressure, diastolic blood pressure, temperature, urinalysis, serum creatinine, blood urea nitrogen, hematocrit, hemoglobin, mean cell volume, mean cell hemoglobin, platelet count, electrolytes, serum albumin, total protein, calcium, phosphorous, electrocardiogram, creatinine phosphokinase, lactate dehydrogenase, alanine aminotransaminase, aspartate aminotransaminase, γ -glutamyl transferase, bilirubin, and alkaline phosphatase. The following safety evaluations were performed before randomization and at the final visit: prothrombin time, partial thromboplastin time, high- and low-density lipoprotein cholesterol, total cholesterol, triglycerides, weight, serum uric acid, and fecal hemoccult. The Scheffé test was used to determine the change in leukocyte count (segmented neutrophils, bands eosinophils, basophils, and monocytes) from baseline to endpoint.

The following ocular safety assessments were performed before randomization and at each subsequent visit: visual acuity (by ETDRS protocol), D15 color testing, visual field 30-2 testing (Humphrey; Carl Zeiss Meditec, Inc., Dublin, CA), clinical cataract assessment, ETDRS retinopathy level (assessed by stereoscopic fundus photography), fluorescein angiography, comprehensive eye examination, intraocular pressure, and contrast sensitivity (Vistech, Dayton, OH). An immunology panel (see the Methodology section) was measured before randomization and at the final visit. Tetanus toxoid was administered 2 weeks after randomization, and the tetanus titer response was mea-

TABLE 1. Patient Demographics by Treatment Group

Therapy	n	Diabetes Type (No. Type 2) (%)	Age (y) Mean (SD) [Range]	Gender (No. F) (%)	Duration of Diabetes (y) Mean (SD) [Range]	Duration of Insulin (y) Mean (SD) [Range]	Insulin Use (n) (%)	BMI (kg/m ²) Mean (SD) [Range]	HbA _{1c} (%) Mean (SD) [Range]
Placebo	7	2 (29)	31.2 (9.5) [20.7-45.3]	2 (29)	4.1 (3.4) [0.7-9.4]	4.2 (3.4) [0.7-9.4]	6 (86)	31 (9.4) [24-47]	8.2 (1.9) [6-10]
8 mg RBX bid	7	2 (29)	42.7 (13.4) [18.3-59.6]	4 (57)	6.5 (2.7) [2.6-9.1]	7.8 (1.4) [5.8-9.1]	5 (71)	30 (1.1) [21-53]	7.9 (1.2) [6-10]
16 mg RBX qd	7	4 (65)	46.1 (12.9) [25.7-65.6]	3 (43)	4.3 (3.5) [0.4-9.6]	3.9 (5) [0.4-9.6]	3 (43)	31 (7.9) [23-47]	7.9 (1.1) [6-9]
16 mg RBX bid	8	3 (38)	43.6 (7.3) [32.5-51.6]	3 (38)	4.8 (3.2) [0.9-8.9]	5.5 (3) [0.9-8.9]	5 (63)	31 (5.2) [23-39]	8.1 (1.7) [6-11]

bid, twice daily; qd, daily.

TABLE 2. Treatment-Emergent Adverse Events (TEAE)

Event Classification	Placebo (n = 7) n (%)	8 mg RBX Bid (n = 7) n (%)	16 mg RBX Qd (n = 7) n (%)	16 mg RBX Bid (n = 8) n (%)	Overall P
Overall					
Patients with ≥ 1 TEAE	7 (100)	6 (85.7)	6 (85.7)	8 (100)	0.586
Patients with 0 TEAE	0	1 (14.3)	1 (14.3)	0	0.586
Specific*					
Abdominal pain	4 (57.1)	1 (14.3)	1 (14.3)	0	0.049
Rhinitis	2 (28.6)	1 (14.3)	4 (57.1)	0	0.051

* Only specific events for which an overall $P < 0.055$ was observed are included.
Bid, twice daily, Qd, daily.

sured at the last visit. Video fluorescein angiographic assessment of mean retinal circulation time and retinal blood flow was performed before randomization and at the final visit.

The trial was conducted in accordance with the Declaration of Helsinki and guidelines for Good Clinical Practice. The protocol was reviewed and approved by the Joslin Diabetes Center institutional review board, and all patients signed a written informed consent form.

Methodology

The University of Wisconsin Fundus Photograph Reading Center confirmed eligible retinopathy during the screening and entry phase. Clinical laboratory measurements were performed at a central laboratory, and immunology testing was performed at Boston Children's Hospital. The immunology panel included counts of total, T, and B lymphocytes; the CD4, CD5, and CD8 lymphocyte subclasses; IgG₁, IgG₂, IgG₃, IgG₄, IgA, IgM, IgE, and IgD levels; hepatitis A titers; and *Neisseria meningococcus* titers.

All patients ingested their initial RBX dose along with a standardized approved dietary allowance (ADA) 1800 calorie per day diet breakfast consisting of 360 calories, 57 g carbohydrate, 12 g protein, and 8 g fat. ECG testing was performed before the initial dose as well as 4, 9, and 24 hours after the dose(s).

Retinal hemodynamic parameters were measured at baseline and study conclusion by dye dilution technique, as previously described²⁶ with a scanning laser ophthalmoscope (model 101; Rodenstock, Munich, Germany) with a 40° field. Video output was digitized in real time at 30 frames per second and stored with a data storage system (TRAPIX Plus/DataStore; Recognition Concepts, Carson City, NV). The analog video signal was simultaneously recorded on a videotape recorder (U-Matic; Sony, Park Ridge, NJ) for archival storage. Digitized images were analyzed on a frame-by-frame basis on a computer (VAX 4000/300; Digital Equipment Corp., Maynard, MA), to determine retinal circulatory parameters, and the resultant data were analyzed as previously described.²⁶

Statistics

Statistical analyses were performed using a two-sided test with a nominal significance level of 0.05 unless otherwise noted. Least-square means were used for all comparisons.

The group comparisons for retinal hemodynamic parameters were performed on computer (SigmaStat; SPSS, Chicago, IL). One-way repeated-measures analysis of variance (ANOVA) was used to compare the same patients at different blood glucose levels. Group comparisons were performed using one-way ANOVA. Populations were tested for normality using the Kolmogorov-Smirnov test for normality and the Levene median test for equality of variance. If the distributions failed the normality or equal variance test, then the Kruskal-Wallis ANOVA on ranks was performed. All pair-wise multiple comparisons were performed with the Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

For pharmacodynamic analyses, a random-effects ANOVA model was used to characterize the dose-response relationship for the response variables (PROC MIXED in SAS, 1989; SAS Institute Inc., Cary, NC). Because it is likely that measurements on the left and right eye of the same person will correlate, a random effects model is appropriate to account for this correlation. The ANOVA model fit to the data is given as: $Y_{ijk} = \mu + D_i + \rho_{j(i)} + \varepsilon_{ijk}$ where: μ is an overall mean, D_i is the treatment effect for dose group i , $i = 1, 2, 3$ (note that the three dose groups were defined as dose = 0 for placebo, dose = 16 for RBX administered as 8 mg twice daily or 16 mg per day, or dose = 32 for RBX per day administered as 16 mg twice daily), $\rho_{j(i)}$ is the random subject effect for patient j in group i (assume $\rho_{j(i)}$ is distributed independently as a normal random variable with mean 0 and variance σ^2), ε_{ijk} is the random error for patient j in group i in the left or right eye k (assume ε_{ijk} is distributed independently as a normal random variable with mean 0 and variance σ^2), and Y_{ijk} is the change from baseline response for patient j in group i in eye k .

In addition, a random-effects analysis of covariance (ANCOVA) model was used to assess the impact of baseline values on the dose-

TABLE 3. Ocular Safety Assessment

Parameter	Placebo n = 7			Pooled RBX n = 22			P
	Better	No Change	Worse	Better	No Change	Worse	
Diabetic retinopathy	1 (14.3)	1 (14.3)	5 (71.4)	2 (9.1)	17 (77.3)	3 (13.6)	>0.999
Clinical lens grading	0	7 (100)	0	2 (9.1)	19 (86.4)	1 (4.5)	>0.999
D15 color	0	6 (85.7)	1 (14.3)	2 (9.1)	17 (77.3)	3 (13.6)	>0.999
Ophthalmic examination	1 (14.3)	5 (71.4)	1 (14.3)	5 (22.7)	10 (45.5)	7 (31.8)	0.528
Visual fields	1 (14.3)	5 (71.4)	1 (14.3)	5 (22.7)	15 (68.2)	2 (9.1)	>0.999
Fluorescein angiography	0	7 (100)	0	0	21 (95.5)	1 (4.5)	>0.999

A patient was classified as better at endpoint if one or both eyes improved and no eye worsened; worse at endpoint if one or both eyes worsened, even if one eye improved; or no change at endpoint if all eyes measured did not change. Probabilities were calculated with the two-tailed Fisher exact test. Data are expressed as the number of subjects (percentage of total group).

TABLE 4. Ocular Contrast Sensitivity

Parameter	Placebo <i>n</i> = 7			RBX <i>n</i> = 22			<i>P</i>
	Better	No Change	Worse	Better	No Change	Worse	
1.5 cyc/deg	6 (85.7)	0	1 (14.3)	11 (50)	9 (40.9)	2 (9.1)	0.100
3 cyc/deg	7 (100)	0	0	7 (31.8)	9 (40.9)	6 (27.3)	0.008
6 cyc/deg	5 (71.4)	2 (28.6)	0	8 (36.4)	7 (31.8)	7 (31.8)	0.208
12 cyc/deg	5 (71.4)	1 (14.3)	1 (14.3)	11 (50)	2 (9.1)	9 (40.9)	0.448
18 cyc/deg	5 (71.4)	1 (14.3)	1 (14.3)	12 (54.5)	4 (18.2)	6 (27.3)	0.840

cyc/deg, cycles per degree. See Table 3 for descriptions of classifications and data.

response relationship. The ANCOVA model fit to the data is given as follows: $Y_{ijk} = \mu + \beta z_{ijk} + D_i + \rho_{j(i)} + \varepsilon_{ijk}$, where z_{ijk} is the baseline pharmacodynamic reading in eye k of patient j in dose group i and β is the slope parameter that relates the baseline value to the change from baseline.

RESULTS

Patient Characteristics

Forty-three patients were evaluated for eligibility, and 29 persons with diabetes mellitus (12 women and 17 men) participated in the study. Eighteen (62%) had type 1 and 11 had type 2 diabetes. Three patients were African-American and the remainder (90%) were white. Average age was 41.0 ± 11.9 years (range, 18.3–65.6), diabetes duration was 4.9 ± 3.2 years (range, 0.4–9.6), HbA_{1c} was $8.0\% \pm 1.4\%$ (range, 6%–11%) and body mass index was 30.7 ± 8.1 kg/m² (range, 21–53). There were no statistically significant differences among treatment groups for any of these parameters at baseline. A summary of patient demographics by treatment group is provided in Table 1.

Safety

Except for abdominal pain, which occurred more frequently in subjects receiving placebo ($P = 0.049$), there were no statistically significant differences among treatment groups in the incidence of treatment-emergent adverse events (Table 2). There was a trend toward more rhinitis in the 16-mg RBX daily group, but it did not reach statistical significance ($P = 0.051$), and no cases were observed in the 16-mg twice daily group. Although not statistically significant, a linear trend toward an increased incidence of all types of pain combined with increasing doses of RBX was observed ($P = 0.08$). Pain of any type was reported as an adverse event in six patients receiving RBX

and in none of the patients receiving placebo. Pain reported by patients receiving RBX was attributed to various sources (e.g., back pain, cramp) without a consistent pattern to suggest a unified underlying pathophysiology. One subject, who received 16 mg RBX daily, reported three hypoglycemic episodes, one of which was severe. Another subject receiving placebo reported one nonsevere hypoglycemic episode. There were no subject deaths during the study.

Of patients receiving RBX, one patient had transient neutropenia without signs or symptoms of an infectious process, and five had transient leukopenia. It was determined with the Scheffé test that the five patients with abnormally low leukocyte counts during the study had significantly lower leukocyte counts than did the other patients at baseline (4.2 billions per liter [GI/L] versus 6.0 GI/L with lower limit of normal being 3.4 to 3.6 GI/L). No statistically significant differences among treatment groups were detected for baseline-to-endpoint changes in total leukocyte counts, segmented neutrophils, bands, eosinophils, basophils, or monocytes. Two RBX-treated patients and one placebo-treated patient had transient low hematocrit values (31%, 37% and 36% with lower limit of normal = 33%, 38%, and 38%, respectively) and one of these RBX-treated patients also had transient low hemoglobin (6.64 mM with lower limit of normal = 6.80 mM) and erythrocyte count ($3.5 \times 10^{12}/L$ with lower limit of normal = $3.7 \times 10^{12}/L$). These low values spontaneously resolved during the course of the study or after the study in one case where the low value occurred only at the final study visit. There was a statistically significant difference between the placebo and 8-mg RBX twice daily groups in the median change from baseline to endpoint in erythrocyte count (+0.1 vs. $-0.1 \times 10^{12}/L$, $P = 0.033$). No other statistically significant differences among treatment groups were detected for baseline-to-endpoint changes in erythrocyte count, hematocrit, or hemoglobin.

TABLE 5. Visual Acuity and Intraocular Pressure Response

Parameter	Group (<i>n</i>)	Baseline Mean (SD)	Endpoint Mean (SD)	Change Mean (SD)	<i>P</i> * (from baseline)	<i>P</i> † (vs. placebo)	<i>P</i> ‡ (linear trend)
Visual acuity (letters correct)	Placebo (14)	59.9 (3.21)	62.5 (4.52)	2.6 (3.39)	0.269		0.843
	16 mg RBX/day (28)	60.0 (2.40)	60.1 (10.9)	0.1 (11.6)	0.949	0.571	
	32 mg RBX/day (16)	60.0 (7.87)	62.0 (7.06)	2.0 (2.19)	0.369	0.969	
Intraocular pressure (mm Hg)	Placebo (12)	13.7 (1.56)	13.1 (3.53)	−0.6 (2.78)	0.480		0.469
	16 mg RBX/day (28)	13.9 (2.32)	13.3 (2.43)	−0.6 (1.71)	0.265	>0.999	
	32 mg RBX/day (16)	14.1 (2.43)	12.7 (2.39)	−1.4 (1.71)	0.062	0.666	

The 8-mg RBX twice daily and 16-mg RBX daily groups were pooled for the trend tests. *n*, number of eyes assessed. IOP measurements from both eyes of one patient were missing.

* Within-group test of a mean difference from zero for change from baseline.

† Between-group comparisons to placebo with the Dunnett-Hsu procedure used to adjust for multiple comparisons.

‡ Tests for linear trend for change from baseline.

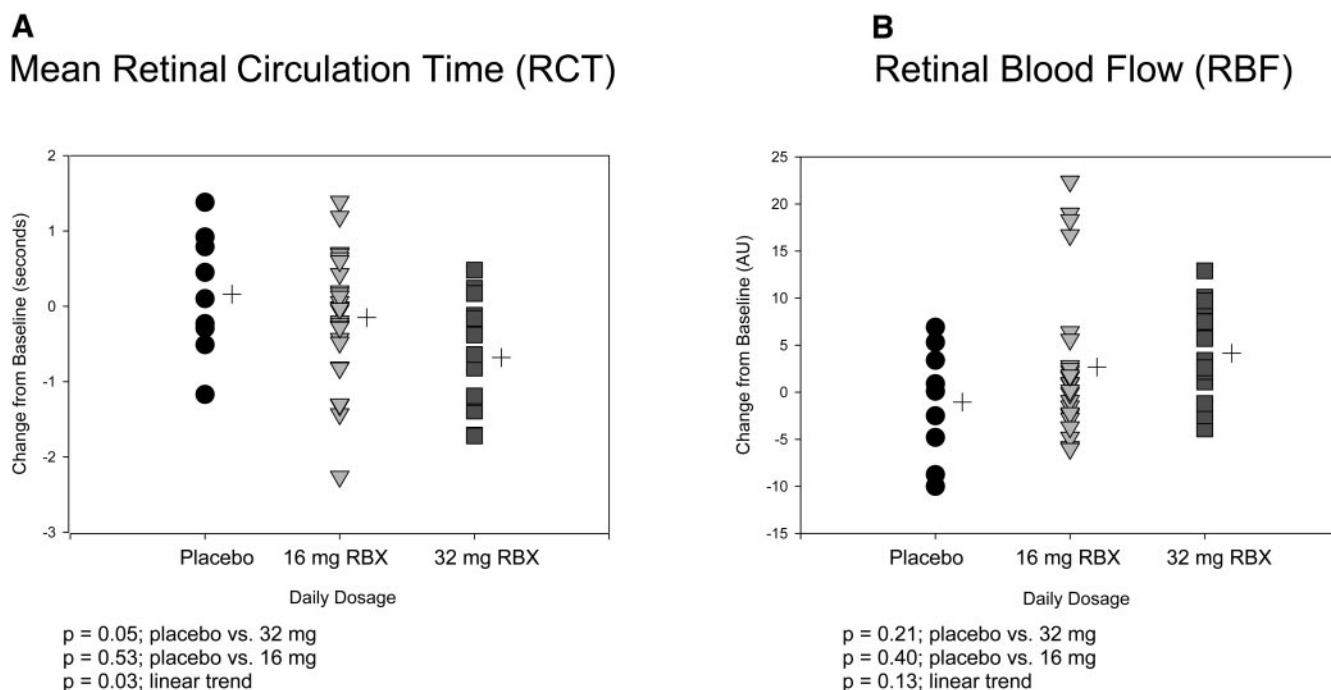


FIGURE 1. Pharmacodynamic effect of RBX on mean RCT (*left*) and retinal blood flow (*right*). Changes from baseline of measurements of mean retinal circulation time (A) and retinal blood flow (B) for each eye are indicated for each RBX dose. The mean value for each group is noted (+). AU, arbitrary units.

The baseline-to-endpoint changes in serum uric acid concentrations were significantly higher in the 16-mg RBX daily and 16-mg RBX twice daily groups (20.4 ± 24.5 and 29.0 ± 23.5 μM , respectively; mean $\pm$ SD) than was observed in the 8-mg RBX twice daily and placebo groups (-32.3 ± 31.4 and -22.1 ± 35.0 μM , respectively; mean $\pm$ SD). However, no abnormally high serum uric acid concentrations were detected in any subject and all mean endpoint serum uric acid concentrations were within the normal reference range (167–547 μM). Creatine phosphokinase levels were elevated in four subjects on one occasion each. None of these subjects reported chest pain or muscle pain at the visits when the enzyme levels were elevated and the elevations were not associated with any ECG abnormalities.

Ocular safety assessments are summarized in Table 3. No statistically significant differences were noted between placebo and RBX for any of these parameters, including AREDS clinical lens grading, D15 color testing, visual field 30-2 testing (Humphrey; Carl Zeiss Meditec), and fluorescein angiography. There was an overall trend toward an increase in fluorescein leakage from baseline to endpoint with increasing RBX dose, as determined by vitreous fluorophotometry ($P = 0.042$). However, there were no statistically significant differences compared with placebo in the change from baseline for any of the RBX doses individually (baseline-to-endpoint results for each dose level were: placebo: 0.78 ± 0.53 arbitrary units (AU) to 0.63 ± 0.93 AU; 16 mg/d: 0.43 ± 0.37 to 0.77 ± 0.60 AU ($P = 0.110$ vs. placebo); 32 mg/d: 0.42 ± 0.56 to 0.39 ± 0.35 AU ($P = 0.856$ vs. placebo). Contrast-sensitivity results (Vistech) were not significantly different between placebo and RBX treatment, except at 3 cyc/deg (Table 4) where the difference was largely driven by improvement in test results in all placebo-treated patients. No changes from baseline were observed in visual acuity or intraocular pressure in patients receiving RBX compared with placebo (Table 5).

There were no statistically significant differences between placebo and RBX treatment in any of the immunology panel

parameters or in the titer response to tetanus toxoid. There were no clinically relevant differences among treatment groups in any electrocardiogram parameters, as evaluated by an expert masked grader. There were no statistically significant differences among treatment groups in any of the other safety assessments listed previously.

Three patients discontinued participation in the study because of adverse events. A subject in the 8-mg RBX twice daily group discontinued after the appearance of neutropenia. A subject in the 16-mg RBX daily group discontinued because of an episode of hypoglycemia. A subject in the placebo group discontinued because of hallucinations.

Pharmacodynamic Effects on Retinal Hemodynamics

In this patient population with no to very mild diabetic retinopathy, retinal mean circulation time (RCT) is prolonged approximately 1 second compared with nondiabetic patients.²⁶ As shown in Figure 1 and Table 6, RBX-treated patients (16 mg twice daily) experienced an RCT baseline-to-endpoint change of -0.68 ± 0.73 seconds compared with a $+0.16 \pm 0.80$ -second change in patients treated with placebo ($P = 0.046$ RBX versus placebo; $P = 0.004$ RBX versus baseline). Baseline-to-endpoint RCT change in the 16 mg/d group was intermediate at -0.15 ± 0.82 seconds ($P = 0.532$ RBX versus placebo). Overall, there appeared to be an RBX dose-dependent effect on RCT ($P = 0.027$ for linear trend). Baseline RCT correlated with baseline-to-endpoint change in RCT ($r = -0.278$, $P = 0.035$). Similar although not statistically significant effects on RBF were observed (Fig. 1B and Table 6) with an increase of 4.14 ± 5.25 AU in the 32-mg RBX per day group compared with a -1.05 ± 5.96 -AU change in patients receiving placebo ($P = 0.211$ RBX versus placebo, $P = 0.043$ RBX versus baseline, $P = 0.133$ for linear trend). Baseline RBF did not correlate with a baseline-to-endpoint change in RBF. Only in patients receiving 16 mg RBX twice daily was arterial diam-

TABLE 6. Retinal Hemodynamic Parameters

Parameter	Group (n)	Baseline Mean (SD)	Endpoint Mean (SD)	Change Mean (SD)	P (from baseline)	P (vs. placebo)	P (linear trend)
Mean retinal circulation time (s)	Placebo (9)	4.72 (0.73)	4.88 (1.11)	0.16 (0.80)	0.597		0.027
	16 mg RBX/day (27)	4.50 (0.97)	4.35 (1.21)	-0.15 (0.82)	0.375	0.532	
	32 mg RBX/day (15)	4.79 (1.00)	4.12 (0.68)	-0.68 (0.73)	0.004	0.046	
Retinal blood flow (AU)	Placebo (9)	35.48 (8.59)	34.43 (8.94)	-1.05 (5.96)	0.762		0.133
	16 mg RBX/day (27)	31.33 (6.48)	34.01 (9.88)	2.68 (7.65)	0.093	0.395	
	32 mg RBX/day (15)	34.81 (9.83)	38.94 (10.27)	4.14 (5.25)	0.043	0.211	
Arterial diameter (pixels)	Placebo (11)	7.52 (0.50)	7.57 (0.56)	0.05 (0.33)	0.445		0.003
	16 mg RBX/day (27)	7.17 (0.59)	7.17 (0.60)	-0.01 (0.17)	0.853	0.648	
	32 mg RBX/day (16)	7.60 (0.72)	7.37 (0.71)	-0.23 (0.22)	<0.001	0.006	

The 8-mg RBX twice daily and 16-mg RBX daily groups were pooled for the trend tests. *n* the number of eyes assessed. Measurements from seven eyes of five patients could not be calculated due to poor image quality.

* Within-group test of a mean difference from zero for change from baseline.

† Between-group comparisons to placebo with the Dunnett-Hsu procedure used to adjust for multiple comparisons.

‡ Tests for linear trend for change from baseline.

eter decreased from baseline to endpoint ($P = 0.003$, Table 6). However, the magnitude of this effect was small (-0.23 ± 0.22 pixels) and less than the 0.50-pixel detection limit for this measurement. No statistically significant differences between the RBX and placebo treatment groups were observed for baseline-to-endpoint changes in retinal vein diameter or dye appearance time.

DISCUSSION

Although data from a subsequent phase-3, multicenter, randomized clinical trial of RBX have recently been published,²⁴ the presently reported study was the first to evaluate RBX pharmacodynamics in humans and the only evaluation to date of the amelioration of diabetes-induced retinal hemodynamic abnormalities by an oral PKC- β inhibitor. Data from this randomized, double masked, parallel, placebo-controlled study in subjects with no or very mild diabetic retinopathy demonstrate that orally administered RBX reaches the retinal microvasculature and is capable of ameliorating the diabetes-induced abnormalities in retinal vascular function. This positive effect on baseline-to-endpoint change in RCT was statistically significant for 32 mg RBX (16 mg twice daily) compared with placebo for mean retinal circulation time. There was also a statistically significant RBX dose-response effect for the same endpoint. In addition, oral administration of RBX was well tolerated in persons with diabetes at doses up to 16 mg twice daily for 28 days. RBX was not associated with an increase in spontaneous adverse event reporting relative to placebo, and no serious safety issues were identified that would preclude continued clinical development of this compound.

The safety profile of a systemically administered compound is of considerable importance, especially when it inhibits a key signaling enzyme such as PKC where substantial toxicity might arise. Indeed, treatment of diabetic patients for 3 months with a multitargeted kinase inhibitor, which also acts as a nonspecific PKC inhibitor, resulted in significant gastrointestinal side effects (nausea, vomiting, and diarrhea) and dose-related problems with glycemic control and liver toxicity. Of interest, the compound also led to reductions in some measures of retinal thickening, as evaluated by optical coherence tomography.²⁷ In contrast, RBX is selective for the β isoform of PKC and highly selective for PKC compared with other kinases.^{6,9,11-13,19} This selectivity for a single PKC isoform that is hypothesized to be involved in mediating diabetes-induced ocular complications may account for the lack of side effects attributable to RBX in this study and in an additional 937 patients treated with placebo or one of three doses of RBX for 30 to 50 months.²⁴

The observed effect of RBX on retinal hemodynamics in the present study is consistent with the hypothesis that hyperglycemia-induced activation of PKC- β may mediate diabetes-induced retinal vascular dysfunction^{18,22} and these are the first human data demonstrating an effect of PKC β inhibition on retinal vascular function. There are considerable data in animal models that support the hypothesis that major diabetic microvascular complications may be mediated through overactivation of PKC- β .^{10,21,28-30}

Recent clinical trial data support this possibility as well, particularly with regard to prevention of visual loss and beneficial effects on macular edema. Although RBX had no effect on the primary endpoint of retinopathy progression in a randomized, placebo-controlled, multicenter clinical trial, RBX treatment for 36 to 46 months reduced the relative risk of moderate visual loss by 63% ($P = 0.012$), with adjustment for potential confounding factors, and reduced the risk of moderate visual loss sustained over 6 months in eyes with definite diabetic

macular edema at baseline from 25% in placebo to 10% in the 32-mg/d RBX-treated group ($P = 0.017$).²⁴ Indeed, a recently completed 3-year, phase-3 randomized, placebo-controlled, multicenter clinical trial evaluating the effect of RBX on preventing sustained moderate visual loss has been initially reported to have met this primary endpoint.³¹ A phase-3 study evaluating the effect of RBX on the progression of diabetic macular edema is ongoing.

Thus, results of these multiple studies suggest that the oral administration of RBX in patients may have positive effects on the nonocular as well as other ocular diabetic microvascular complications, such as macular edema and nephropathy, previously shown to be mediated by PKC- β in animals.^{10,21,28–30} If such activity is substantiated in clinical trials primarily assessing these endpoints, selective inhibition of PKC isozymes may offer a unique therapeutic approach to retinopathy and other diabetes-related microvascular disorders.

Acknowledgments

The authors thank Kirk Ways and Carlos Alatorre for providing valuable insight into the design of the study; Louis Vignati and Mary Jo Vodnick for their leadership throughout the development and conduct of the study; Ann Kopple for outstanding study coordination; Pamela Beaumont for invaluable patient care assistance; and Keri Kles, Mary Anderson, and Jerry Cavallerano for help with the manuscript.

References

- Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977–986.
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33): UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998;352:837–853.
- Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*. 1995;28:103–117.
- Inoguchi T, Battan R, Handler E, et al. Preferential elevation of protein kinase C isoform β II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci USA*. 1992;89:11059–11063.
- Xia P, Inoguchi T, Kern TS, et al. Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. *Diabetes*. 1994;43:1122–1129.
- Shiba T, Inoguchi T, Sportsman JR, et al. Correlation of diacylglycerol level and protein kinase C activity in rat retina to retinal circulation. *Am J Physiol*. 1993;265:E783–E793.
- Craven PA, DeRubertis FR. Protein kinase C is activated in glomeruli from streptozotocin diabetic rats: possible mediation by glucose. *J Clin Invest*. 1989;83:1667–1675.
- Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000;404:787–790.
- Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes*. 1998;47:859–866.
- Cotter MA, Jack AM, Cameron NE. Effects of the protein kinase C β inhibitor LY333531 on neural and vascular function in rats with streptozotocin-induced diabetes. *Clin Sci*. 2002;103:311–321.
- Yan SF, Lu J, Zou YS, et al. Protein kinase C- β and oxygen deprivation: a novel Egr-1-dependent pathway for fibrin deposition in hypoxemic vasculature. *J Biol Chem*. 2000;275:11921–11928.
- Nagpala PG, Malik AB, Vuong PT, Lum H. Protein kinase C β 1 overexpression augments phorbol ester-induced increase in endothelial permeability. *J Cell Physiol*. 1996;166:249–255.
- Wolf BA, Williamson JR, Easom RA, et al. Diacylglycerol accumulation and microvascular abnormalities induced by elevated glucose levels. *J Clin Invest*. 1991;87:31–38.
- Xia P, Aiello LP, Ishii H, et al. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest*. 1996;98:2018–2026.
- Funatsu H, Yamashita H, Ikeda T, et al. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. *Ophthalmology*. 2003;110:1690–1696.
- Suzuma K, Takahara N, Suzuma I, et al. Characterization of protein kinase C β isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. *Proc Natl Acad Sci USA*. 2002;99:721–726.
- Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med*. 1994;331:1480–1487.
- Aiello LP, Bursell SE, Clermont A, et al. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective β -isoform-selective inhibitor. *Diabetes*. 1997;46:1473–1480.
- Jirousek MR, Gillig JR, Gonzalez CM, et al. (S)-13-[(dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9,16,21-dimetheno-1H,13H-dibenzo[e,k]pyrrolo[3,4-H][1,4,13]oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) and related analogues: isozyme selective inhibitors of protein kinase C β . *J Med Chem*. 1996;39:2664–2671.
- Engel GL, Farid NA, Faul MM, Richardson LA, Winneroski LL. Salt form selection and characterization of LY333531 mesylate monohydrate. *Int J Pharm*. 2000;198:239–247.
- Ishii H, Jirousek MR, Koya D, et al. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC β -inhibitor. *Science*. 1996;272:728–731.
- Danis RP, Bingaman DP, Jirousek M, Yang Y. Inhibition of intraocular neovascularization caused by retinal ischemia in pigs by PKC β inhibition with LY333531. *Invest Ophthalmol Vis Sci*. 1998;39:171–179.
- Nonaka A, Kiryu J, Tsujikawa A, et al. PKC β inhibitor (LY333531) attenuates leukocyte entrapment in retinal microcirculation of diabetic rats. *Invest Ophthalmol Vis Sci*. 2000;41:2702–2706.
- The PKC-DRS Study Group. The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: initial results of the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS) multicenter randomized clinical trial. *Diabetes*. 2005;54:2188–2197.
- Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy. ETDRS report number 12. *Ophthalmology*. 1991;98:823–833.
- Bursell SE, Clermont AC, Kinsley BT, et al. Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 1996;37:886–897.
- Campochiaro PA, C99-PKC412-003 Study Group. Reduction of diabetic macular edema by oral administration of the kinase inhibitor PKC412. *Invest Ophthalmol Vis Sci*. 2004;45:922–931.
- Koya D, Haneda M, Nakagawa H, et al. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC β inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J*. 2000;14:439–447.
- Kelly DJ, Zhang Y, Hepper C, et al. Protein kinase C β inhibition attenuates the progression of experimental diabetic nephropathy in the presence of continued hypertension. *Diabetes*. 2003;52:512–518.
- Aiello LP. The potential role of PKC β in diabetic retinopathy and macular edema. *Surv Ophthalmol*. 2002;47:S263–S269.
- Eli Lilly Press Release. Available at <http://newsroom.lilly.com/ReleaseDetail.cfm>. Accessed August 26, 2005.