ORAL PROTEIN KINASE C β INHIBITION USING RUBOXISTAURIN

Efficacy, Safety, and Causes of Vision Loss Among 813 Patients (1,392 Eyes) with Diabetic Retinopathy in the Protein Kinase C β Inhibitor-Diabetic Retinopathy Study and the Protein kinase C β Inhibitor-Diabetic Retinopathy Study 2

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Purpose: To evaluate efficacy, safety, and causes of vision loss among 813 patients (1,392 eyes) with moderately severe to very severe nonproliferative diabetic retinopathy from the Protein Kinase C β Inhibitor-Diabetic Retinopathy Study and Protein Kinase C β Inhibitor-Diabetic Retinopathy Study 2 ruboxistaurin (RBX) protein kinase C β inhibitor trials.

Methods: Patients in these 3-year, randomized, placebo-controlled, double-masked, Phase 3 trials had best-corrected Early Treatment Diabetic Retinopathy Study visual acuity ≥45 letters (~20/125 Snellen), Early Treatment Diabetic Retinopathy Study retinopathy level 47A/B-53E, and no previous panretinal photocoagulation in ≥1 eye. Patients received placebo (N = 401) or RBX 32 mg/day (N = 412). Data from the 2 studies were combined and masked evaluation of retinal photographs was performed for cause of visual decline in all patients experiencing sustained moderate visual loss (≥15-letter loss sustained for the last 6 months of study).

Results: In the studies combined, sustained moderate visual loss occurred in 10.2% of placebo-treated patients versus 6.1% of RBX-treated patients ($P = 0.011$). A ≥15-letter gain occurred in 2.4% of placebo versus 4.7% of RBX eyes ($P = 0.021$) and a ≥15-letter loss occurred in 11.4% versus 7.4%, respectively ($P = 0.012$). Diabetic macular edema was the probable primary cause of vision loss. Among eyes without focal/grid photocoagulation at baseline, fewer RBX group eyes (26.7%) required initial focal/grid photocoagulation versus placebo (35.6%; $P = 0.008$). No safety concerns were identified.

Conclusion: Analysis of data combined from two similar studies adds further statistical significance to RBX's beneficial effects on visual loss, need for focal laser, and vision gain, most likely through effects on macular edema.

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In the United States, diabetic retinopathy (DR) results in 12,000 to 24,000 new cases of blindness each year, or approximately 30 to 70 cases per day, making it the leading cause of new-onset blindness among adults aged 20 years to 74 years.[1] The molecular mechanism by which diabetes damages the retinal microvasculature is thought to be multifactorial, with possible roles for hyperglycemia-induced polyol pathway activation, production of advanced glycation end products, oxidative stress, and activation of the diacylglycerol–protein kinase C (PKC) transcription pathway.[2–4]

Ruboxistaurin (RBX), an orally administered, isoform-selective inhibitor of PKC β, has been shown to

have a beneficial effect in animal models of DR[5–8] and has also been shown to ameliorate diabetes-induced retinal hemodynamic abnormalities in patients with diabetes.[9]

The 3-year, multidose, parallel, randomized, double-masked, placebo-controlled, Phase 3 Protein Kinase C β Inhibitor-Diabetic Retinopathy Study (PKC-DRS) demonstrated that although RBX (32 mg/day) had no effect on the primary endpoint of progression of nonproliferative diabetic retinopathy (NPDR) to proliferative DR in patients who had moderately severe to very severe NPDR at baseline, it did delay the time to occurrence of moderate visual loss (a loss of ≥15 letters on the Early Treatment Diabetic Retinopathy Study [ETDRS] eye chart).[10] Possible explanations for these outcomes are discussed in detail in the initial report from the study.[10] The Protein Kinase C β Inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS2) was a subsequent 3-year, parallel, randomized, double-masked, placebo-controlled, Phase 3 study in patients with moderately severe to very severe NPDR, which demonstrated that RBX-treated patients experienced significantly less sustained moderate visual loss (SMVL, defined as moderate visual loss sustained during the last 6 months of study participation) than placebo-treated patients (5.5% vs. 9.1%; 40% risk reduction; $P = 0.034$).[11]

Sustained moderate visual loss was the primary outcome in the PKC-DRS2 and a secondary outcome in the PKC-DRS. The combined analysis presented here was not prospectively defined.

We now report combined results derived from all 813 patients (1,392 eyes) receiving either placebo or RBX 32 mg/day in these 2 similarly designed studies. The effect of RBX on SMVL, mean and categorical measures of visual acuity, application of focal/grid photocoagulation (FPC), DR progression or application of panretinal photocoagulation, and adverse events from the combined data set is compared with the results of the individual studies. Moreover, the probable causes of vision loss in study eyes of patients who experienced SMVL were specifically assessed by masked fundus photography review and are described for both treatment groups.

These new analyses further support a beneficial effect of RBX on vision loss, reduced need for initial FPC, and increased frequency of vision gain and suggest that these outcomes are mediated primarily through an effect on diabetic macular edema (DME).

Patients and Methods

For both studies, entry criteria included a diagnosis of Type 1 or 2 diabetes, age ≥18 years, hemoglobin A1c (HbA1c) ≤13%, and blood pressure <180/105 (PKC-DRS) or <190/105 (PKC-DRS2). Eye eligibility criteria for both studies included best-corrected ETDRS visual acuity ≥45 letters (approximately 20/125 Snellen or better); moderately severe to very severe NPDR according to the ETDRS severity scale assessed in retinal photographs at a central reading center (retinopathy levels 47B-53E in the PKC-DRS and 47A-53E in the PKC-DRS2,[12] equivalent to moderate to severe NPDR on the American Academy of Ophthalmology severity scale);[13] no glaucoma or current vitreous or preretinal hemorrhage; and no previous panretinal photocoagulation. There were no restrictions on the presence of DME or previous FPC at baseline. Patients were required to have at least one eligible eye. If the fellow eye had proliferative DR or a history of panretinal photocoagulation at baseline, then only the eligible eye was considered a study eye. Otherwise, both eyes were considered study eyes. Patients were allowed to receive FPC and/or panretinal photocoagulation in either eye postbaseline, at the discretion of the investigator.

The design and methods for both studies have been previously reported.[10,11] Briefly, both were multicenter, parallel, placebo-controlled, double-masked, Phase 3 clinical trials. Patients in the PKC-DRS were randomly assigned to receive oral placebo (N = 61), RBX 8 mg/day (N = 60), RBX 16 mg/day (N = 64), or RBX 32 mg/day (N = 67) and were followed-up for 36 months to 46 months. Patients in the PKC-DRS2 were

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Combined analyses from these studies presented in part at the American Diabetes Association's 66th Scientific Sessions (Washington, DC, 2006), the 42nd European Association for the Study of Diabetes Annual Meeting (Copenhagen-Malmoe, Denmark, 2006), and the International Diabetes Federation 19th World Diabetes Congress (Cape Town, South Africa, 2006), and published in abstract form (*Diabetes* 2006;55 Suppl 1:230 OR).

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The members of the PKC-DRS Study Group and PKC-DRS2 Study Group are listed in the Appendix.

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randomly assigned to receive oral placebo (N = 340) or RBX 32 mg/day (N = 345), and were followed-up for 36 months to 42 months.

An ophthalmologic examination (including slit-lamp biomicroscopy, intraocular pressure, and ophthalmoscopy) was performed, and best-corrected visual acuity was measured[14] at screening and at each follow-up visit (every 3 months over a period of 2 [PKC-DRS] or 3 [PKC-DRS2] years, and then every 6 months thereafter). Examiners were certified for both refraction and visual acuity determination. The visual acuity lane, equipment, and lighting were inspected, certified, and monitored by the EMMES Corporation (Rockville, MD). Stereoscopic color fundus photographs were obtained on film at baseline, at the 3-month and 6-month visits, and every 6 months thereafter.[15] Optical coherence tomography was not carried out in either study, in part because the original outcome variable in both studies was worsening of DR severity, not development or worsening of DME. In addition, at the time the first study was initiated (1998), optical coherence tomography devices had not yet become widely adopted in clinical practice.

After the studies were completed, evaluation of probable causes of vision loss was performed for all study eyes that developed SMVL. One of us (M. D. D.), who was masked to treatment assignment, carried out a longitudinal review of all fundus photographs, photograph gradings, and visual acuity scores from baseline and follow-up visits of all patients who experienced SMVL. For each study eye with SMVL, one or more of the following probable primary causes of vision loss was assigned: 1) DME involving the center of the macula, 2) center-involved DME and cataract, 3) cataract, 4) vitreous hemorrhage, 5) severe proliferative DR, 6) vascular occlusion, or 7) undetermined. Center-involved DME was considered to be the probable cause of SMVL when the loss occurred after the development of center-involved DME, when it was not present at baseline, or after it had been present for several visits and no other cause was apparent. The duration of center-involved DME before the occurrence of SMVL varied a great deal, from fewer than 3 months to ≥2 years. In some cases, a ≥15-letter loss occurred only after retinal thickening at the center of the macula was no longer visible. In keeping with long-standing clinical experience, in such cases the loss was attributed to DME if no other cause was apparent. Attribution of SMVL to cataract was based on concordance between change in visual acuity and change in fundus reflex photographs and/or visibility of the retina during follow-up. Initial results of the PKC-DRS, and previous clinical experience, suggested that retinal thickening and/or associated hard exudates at the center of the macula were likely causes of the visual decline observed in these trials. The longitudinal review for probable cause of SMVL, although not specified in the protocol, was undertaken to test the validity of this presumption and to determine whether there was a difference by treatment assignment. In view of this limited goal, review by a single observer masked to treatment assignment but without replication was deemed adequate.

At each study visit, patients were asked regarding the occurrence of adverse events. Treatment-emergent adverse events were defined as those that were not present before initiation of study drug or those that were present before initiation of study drug but worsened either in intensity or frequency after exposure to study drug. Serious adverse events were defined as those that resulted in death, hospitalization, life-threatening consequences, severe or permanent disability, cancer, or other significant consequence.

Placebo and RBX groups were compared using the following statistical tests: chi-square or Fisher exact test for categorical baseline characteristics, reasons for discontinuation, application of FPC, probable causes of vision loss, and adverse events; analysis of variance for continuous baseline characteristics and mean change from baseline in visual acuity by visit; Cochran-Mantel–Haenszel test of proportions, stratified by number of study eyes (one vs. two) and severity of DME, for the occurrence of SMVL; logistic regression of the occurrence of SMVL (generalized estimation equation was applied to take into account the correlation between two eyes from the same patient); and Wilcoxon–Mann-Whitney test and chi-square test for categorical analyses of change in visual acuity from baseline to endpoint.

To evaluate the effect of 32 mg of RBX on SMVL across 2 very similar studies of RBX in patients with DR, a combined analysis consisting of a Cochran–Mantel–Haenszel test similar to that specified as the primary efficacy analysis for the PKC-DRS2 study was performed. This analysis used the stratification scheme from the PKC-DRS2 study (baseline DME severity and number of study eyes) plus a stratum for study. For all analyses, P values ≤0.05 (2-sided) were considered significant.

Both studies, the PKC-DRS and PKC-DRS2, were conducted in accordance with the principles of the Declaration of Helsinki. An institutional review or ethics board approved study conduct at each center, and investigators obtained written informed consent before conducting any study-related procedure.

Table 1. Baseline Demographic and Ocular Characteristics

	Studies Combined		PKC-DRS		PKC-DRS2	
	Placebo Patients (N = 401)	RBX Patients (N = 412)	Placebo Patients (N = 61)	RBX Patients (N = 67)	Placebo Patients (N = 340)	RBX Patients (N = 345)
Age, years	59 ± 11	59 ± 11	56 ± 14	56 ± 12	59 ± 11	59 ± 11
Range, years	22–84	23–87	22–84	29–82	26–82	23–87
DM type, % Type 2	86	88	77	84	88	88
Sex, % men	65	64	70	73	64	62
Origin, % white	81	75	84	76	80	75
DM duration, years	16 ± 8	16 ± 8	17 ± 7	16 ± 8	16 ± 8	16 ± 8
Range, years	0.2–42.2	0.2–51.4	0.9–36.1	1.8–35.8	0.22–42.2	0.16–51.4
BMI, kg/m^2	32 ± 7	32 ± 8	31 ± 5	31 ± 6	33 ± 7	33 ± 8
HbA1c, %	8.2 ± 1.4	8.2 ± 1.4	8.8 ± 1.3	8.7 ± 1.4	8.1 ± 1.4	8.1 ± 1.4
Range, %	4.9–12.7	5.3–13.0	6.0–12.1	6.1–13.0	4.9–12.7	5.3–12.5
Systolic BP, mmHg	139 ± 18	136 ± 17	142 ± 19	138 ± 18	138 ± 18	136 ± 17
Diastolic BP, mmHg	78 ± 10	78 ± 11	81 ± 10	80 ± 10	77 ± 10	78 ± 11
ACEI use, % yes	53	51	38	45	55	52
Insulin use, % yes	57	59	46	46	55	58
Antihypertensive use, % yes	78	75	66	64	80	77
Patients with 2 study eyes, n (%)	283 (70.6)	296 (71.8)	39 (63.9)	42 (62.7)	244 (71.8)	254 (73.6)
Number of study eyes	684	708	100	109	584	599
ETDRS severity <47, n (% eyes)*	62 (9.1)	72 (10.2)	9 (9.0)	10 (9.2)	53 (9.1)	62 (10.4)
ETDRS severity 47A, n (% eyes)*	245 (35.8)	252 (35.6)	10 (10.0)	8 (7.3)	235 (40.2)	244 (40.7)
ETDRS severity 47B-D, n (% eyes)	152 (22.2)	173 (24.4)	34 (34.0)	50 (45.9)	118 (20.2)	123 (20.5)
ETDRS severity 53, n (% eyes)	222 (32.5)	209 (29.5)	44 (44.0)	39 (35.8)	178 (30.5)	170 (28.4)
ETDRS severity >53, n (% eyes)*	1 (0.1)	2 (0.3)	1 (1.0)	2 (1.8)	0 (0.0)	0 (0.0)
Best-corrected visual acuity, ETDRS letters correct	77.7 ± 11.6	77.7 ± 12.1	79.4 ± 13.1	80.4 ± 9.4	77.4 ± 11.4	77.2 ± 12.5
Previous FPC (% eyes yes)	319 (46.6)	304 (42.9)	28 (28.0)	34 (31.2)	291 (49.8)	270 (45.1)
No DME to minimal DME, n (% eyes)†	167 (24.4)	177 (25.0)	29 (29.0)	36 (33.3)	138 (23.7)	141 (23.6)
Non-CSME, n (% eyes)†	140 (20.5)	119 (16.8)	21 (21.0)	12 (11.1)	119 (20.4)	107 (17.9)
Non–center-involved CSME, n (% eyes)†	151 (22.1)	172 (24.3)	23 (23.0)	32 (29.4)	128 (22.0)	140 (23.5)
Center-involved CSME, n (% eyes)†	224 (32.7)	237 (33.5)	27 (27.0)	28 (25.7)	197 (33.8)	209 (35.0)

Data are presented as percentages, ranges, or means ± SDs. There were no significant between-group differences for any of these baseline characteristics, in either the studies combined or in the individual studies.

*For study eligibility, DR had to be within a specified ETDRS severity range in at least 1 eye (PKC-DRS: 47B-53E; PKC-DRS2: 47A-53E). Fellow eyes with less severe DR were also included as study eyes if they met other eligibility criteria. Three eyes with proliferative DR (ETDRS severity >53) were inappropriately randomized in the PKC-DRS, but were included in subsequent analyses for intent-to-treat purposes.

†No DME to minimal DME refers to eyes with no DME, plus eyes with DME <1/6 disk area. Non-CSME refers to eyes with DME >1/6 disk area, >500 μm from the center of the macula (but without a locus of DME ≥1 disk area in size that extends to within 1 disk diameter of the center of the macula). Non–center-involved CSME refers to eyes with DME ≥1 disk area, with the posterior edge ≤1,500 μm from the center of the macula, plus eyes with any DME ≤500 μm but >100 μm from the center of the macula. Center-involved DME refers to eyes with DME at or within 100 μm of the center of the macula.

ACEI, angiotensin-converting enzyme inhibitor; BP, blood pressure; BMI, body mass index; CSME, clinically significant macular edema; DM, diabetes mellitus.

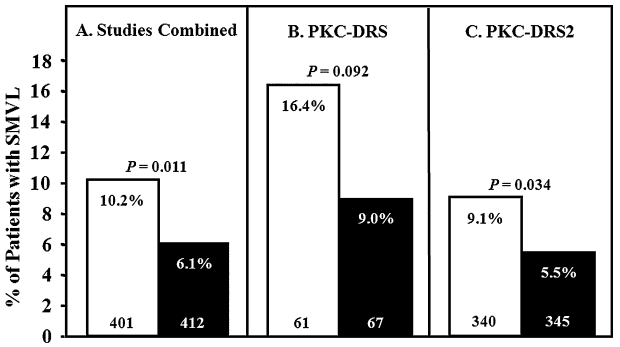

Fig. 1. Percent of placebo-treated (□) and RBX-treated (■) patients who experienced SMVL in (**A**) the studies combined, (**B**) the PKC-DRS, and (**C**) the PKC-DRS2. The total number of patients per group is shown at the bottom within each bar. P values correspond to the difference between treatment groups.

Results

The analyses include a total of 401 placebo-treated patients and 412 RBX-treated patients (32 mg/day) who participated in the similarly designed PKC-DRS (N = 61 placebo and 67 RBX patients) and PKC-DRS2 (N = 340 placebo and 345 RBX patients). In the combined analysis, there were no significant differences between the placebo and RBX groups with regard to baseline demographic or ocular characteristics (Table 1). Patients were predominantly men and white, with Type 2 diabetes, 58.8 ± 11.3 years of age (range, 21.5–86.7 years), HbA1c of 8.2% ± 1.4% (range, 4.9–13.0%), and duration of diabetes of 16.1 ± 8.1 years (range, 0.2–51.4 years) (mean ± SD). Approximately 70% of patients had 2 study eyes. Over one-half of study eyes had clinically significant macular edema, as defined by the ETDRS Study Group,[15] at baseline, and one third of study eyes had DME at or within 100 μm of the center of the macula. In the entire group, approximately 45% of study eyes had received FPC before baseline (Table 1). In PKC-DRS2, the percent of individuals with previous FPC was 49.8% for placebo-treated patients and 45.1% for RBX-treated patients.

Overall, SMVL occurred in 10.2% (41/401) of placebo-treated patients versus 6.1% (25/412) of RBX-treated patients (41% risk reduction, $P = 0.011$; Cochran-Mantel–Haenszel test of proportions) (Figure 1A). Similar risk reductions for SMVL were observed in the PKC-DRS (45% risk reduction; $P = 0.092$; Cochran-Mantel–Haenszel test of proportions) and the PKC-DRS2 (40% risk reduction, $P = 0.034$; Cochran-Mantel–Haenszel test of proportions), as shown in Figure 1, B and C, respectively.

Categorical analyses of change in visual acuity from baseline to endpoint are shown in Table 2. In the entire group, approximately twice as many eyes of RBX-treated patients gained ≥15 letters of visual acuity compared with eyes of placebo-treated patients (4.7% vs. 2.4%, $P = 0.021$; chi-square test). In addition, approximately one-third fewer eyes of RBX-treated patients lost ≥15 letters of visual acuity (7.4% vs. 11.4%; $P = 0.012$; chi-square test). These effects were similar to those observed for the individual studies (Table 2). The overall difference between treatment groups in these categorical analyses was highly significant ($P = 0.001$; Wilcoxon–Mann–Whitney test). Additionally, treatment with RBX 32 mg/day led to a significant improvement ($P = 0.005$; Pearson chi-square test) in the baseline-to-endpoint change in visual acuity in DR study eyes with baseline visual acuity <74 letters in both studies combined (Figure 2).

Logistic regression was performed to evaluate the effect of covariates on SMVL and to ensure that the treatment effect could not be attributed to other factors. Model selection was carried out using ordinary logistic regression at the eye level with the following variables (at baseline): treatment (RBX vs. placebo), age (≥55 vs. <55 years), sex (male vs.

Table 2. Categorical Analyses of Change in Visual Acuity from Baseline to Endpoint

Change in Visual Acuity*, n (%)	Studies Combined			PKC-DRS			PKC-DRS2		
	Placebo Eyes (N = 666)	RBX Eyes (N = 676)	Between-Group P†	Placebo Eyes (N = 93)	RBX Eyes (N = 105)	Between-Group P‡	Placebo Eyes (N = 573)	RBX Eyes (N = 571)	Between-Group P**
≥15-letter gain	16 (2.4)	32 (4.7)	0.021	2 (2.2)	4 (3.8)	0.497	14 (2.4)	28 (4.9)	0.027
<15-letter loss to <15-letter gain	574 (86.2)	594 (87.9)	0.359	72 (77.4)	89 (84.8)	0.186	502 (87.6)	505 (88.4)	0.665
≥15-letter loss	76 (11.4)	50 (7.4)	0.012	19 (20.4)	12 (11.4)	0.082	57 (9.9)	38 (6.7)	0.044

*From baseline to endpoint, measured as ETDRS letters correct. The overall pattern (RBX effective for letter gain and against letter loss) was significant.

†$P = 0.001$, ‡$P = 0.07$, **$P = 0.005$.

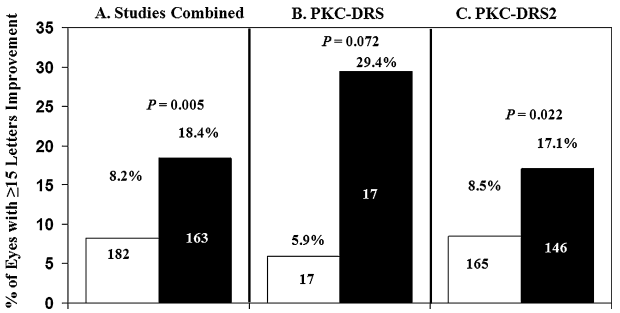

Fig. 2. Baseline-to-endpoint (last observation carried forward) visual acuity change in DR study eyes with baseline visual acuity <74 (ETDRS letters correct) in (A) the studies combined, (B) the PKC-DRS, and (C) the PKC-DRS2 in placebo-treated (□) and RBX-treated (■) patients. The total number of eyes per group is shown at the bottom within each bar. *P* values correspond to the difference between treatment groups.

female), HbA1c (≥8% vs. <8%), ETDRS DR severity (>47A vs. ≤47A; and <53 vs. ≥53), DME severity (eyes with DME ≤500 μm from the center of the macula vs. eyes with either no DME or DME >500 μm from the center of the macula); visual acuity (≥85 vs. <85 ETDRS letters correct), antihypertensive use (yes vs. no); previous FPC (yes vs. no), systolic blood pressure (≥130 vs. <130 mmHg), diastolic blood pressure (≥80 vs. <80 mmHg), body mass index (≥30 vs. <30 kg/m^2), insulin use (yes vs. no), study (PKC-DRS2 vs. PKC-DRS), and treatment by study interaction. The final 2 variables, study and treatment by study interaction, were included to evaluate the disparity between the 2 studies with regard to the frequency of occurrence of SMVL, as SMVL occurred at a greater rate in the PKC-DRS as compared with the PKC-DRS2. The treatment by study interaction term was not significant and was therefore omitted from the final model. The study variable neared statistical significance and was retained in the final model. After excluding variables that did not reach or nearly reach statistical significance in the full model, a reduced model was fitted using a generalized estimation equation analysis, which accounted for possible correlation between two study eyes of the same person, using all study eyes with complete covariate data. For 24 eyes missing baseline HbA1c, we used the HbA1c value from the visit nearest to baseline and found the results to be equivalent whether these eyes were included or not. Odds ratio (OR) estimates, 95% confidence intervals (CI), and *P* values from the generalized estimation equation model including the imputed HbA1c values are shown in Figure 3.

After adjustment for the final factors included in the model (listed in Figure 3), RBX treatment significantly reduced the risk of SMVL relative to placebo (OR, 0.49; 95% CI, 0.29-0.83; $P = 0.008$). Early Treatment Diabetic Retinopathy Study DR severity >47A (OR, 2.27; 95% CI, 1.30–3.98; $P = 0.004$) and DME ≤500 μm from the center of the macula (OR, 4.80; 95% CI, 2.39–9.62; $P < 0.0001$) at baseline were significantly associated with an increased risk of SMVL, as was HbA1c ≥8% at baseline (OR, 1.82; 95% CI, 1.04–3.19; $P = 0.035$). Inclusion in the PKC-DRS2, as compared with the PKC-DRS, trended toward significant association with reduced risk of SMVL (OR, 0.57; 95% CI, 0.29–1.10; $P = 0.095$).

Mean visual acuity by visit in study eyes of placebo-treated versus RBX-treated patients in the studies combined is shown in Figure 4. Visual acuity in the RBX group was consistent throughout the entire study, never declining significantly from baseline, and the difference between the RBX and placebo groups was significant from 12 months onward. Over 3 years, eyes of placebo-treated patients lost a mean of 3.4 letters, as compared with a loss of 1.4 letters in eyes of RBX-treated patients, resulting in a mean treatment

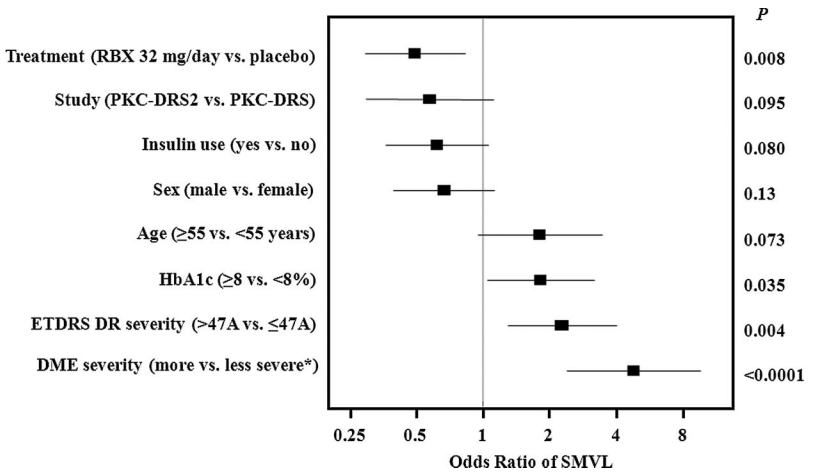

Fig. 3. Logistic regression analysis (generalized estimation equation) of SMVL in study eyes. "More severe" (asterisk) refers to eyes with DME ≤500 μm from the center of the macula at baseline, while "less severe" refers to eyes with either no DME or DME >500 μm from the center of the macula at baseline. The vertical gray line represents an OR = 1. Odds ratios <1 indicate that the first group noted on the left has a lower risk of SMVL, while ORs >1 indicate that the first group listed has a greater risk of SMVL. An OR = 1 indicates identical risk of SMVL between groups.

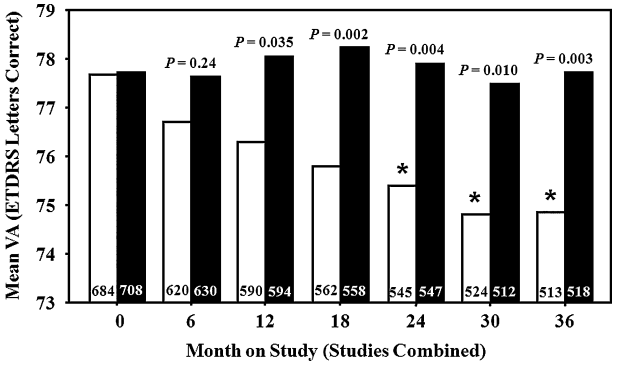

Fig. 4. Mean visual acuity (ETDRS letters correct) at each visit for study eyes of placebo-treated (□) and RBX-treated (■) patients in the studies combined. The total number of eyes per group is shown at the bottom within each bar. *P* values correspond to the difference between treatment groups at each visit. Asterisk denotes a statistically significant decrease from baseline in mean visual acuity. For the studies combined, the mean baseline-to-endpoint change in visual acuity (last observation carried forward) was −3.4 versus −1.4 letters in the placebo and RBX groups, respectively ($P = 0.006$; analysis of variance).

benefit of 2.0 ETDRS letters (last observation carried forward; $P = 0.006$ between groups; analysis of variance). The mean RBX treatment effects observed over 3 years in the individual studies were similar to those observed in the studies combined (last observations carried forward: PKC-DRS: 3.5 ETDRS letters, $P = 0.16$ between groups; PKC-DRS2: 1.8 ETDRS letters, $P = 0.014$ between groups; analysis of variance).

Although there were no significant between-treatment group differences in the application of FPC among all eyes (Group 1, Figure 5), RBX eyes without FPC before baseline were significantly less likely to require initial FPC as compared with placebo, in both the studies combined and in the PKC-DRS2 (Group 2, Figure 5). Overall, initial FPC was performed in 26.7% (108/404) of RBX eyes, as compared with 35.6% (130/365) of placebo eyes ($P = 0.008$; chi-square test).

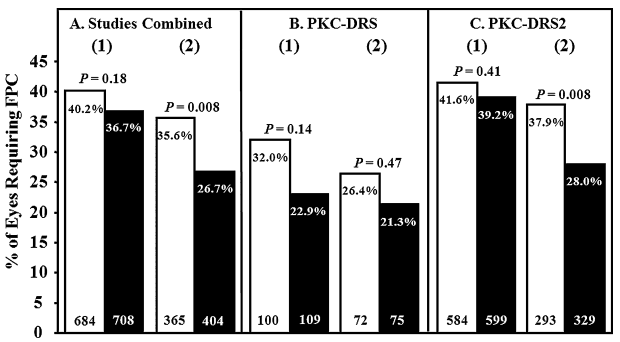

Fig. 5. (1) Percent of all eyes that required FPC; and (2) percent of eyes without FPC before baseline that required initial application of FPC, during (**A**) the studies combined, (**B**) the PKC-DRS, and (**C**) the PKC-DRS2. The total number of eyes per group is shown at the bottom within each bar. P values correspond to the difference between treatment groups.

RBX had no demonstrable effect on DR progression by 3(2) steps on the ETDRS person (eye) scale or application of panretinal photocoagulation, in any of the analyses (data not shown).

Results of the review of fundus photographs are shown in Table 3. Center-involved DME was considered to be the probable cause of SMVL in 72.2% to 100.0% of study eyes in placebo-treated patients and in 60.0% to 83.3% of study eyes in RBX-treated patients. In each analysis, DME accounted for a higher percentage of vision loss in the placebo group than in the RBX group, although these differences were not statistically significant. No other presumed cause of visual loss was observed in >4 eyes in any group, nor associated with greater than approximately 16% of any group's visual loss (and generally much less). Fluorescein angiograms were not obtained in these studies. Consequently, the contribution of macular nonperfusion to the development of SMVL cannot be determined apart from its presumed presence in the 4 eyes in which retinal vascular occlusion was the apparent cause of SMVL.

Considering just the RBX 32-mg/day dose, 412 patients took RBX for periods of up to 4 years, representing 1,074 patient-years of exposure. Three hundred and seventy patients were treated with RBX for over 6 months, and 356 patients were treated with RBX for over 1 year. Adverse events were consistent with those reported in previous studies.[9–11,16–18] Forty-one deaths occurred overall (placebo: 5.7% [23/401]; RBX: 4.4% [18/412]; $P = 0.37$; chi-square test), none of which were considered by the principal investigators to be related to study drug. For both the placebo and RBX groups, approximately 40% of patients experienced ≥1 serious adverse event ($P = 0.30$; Fisher exact test), and >90% of patients experienced ≥1 treatment-emergent adverse event ($P = 0.11$; Fisher exact test). Approximately one fourth of patients in each treatment group discontinued. The reasons for discontinuations were similar between groups (Table 4).

Overall, adverse events that occurred significantly more often in the RBX group, as compared with placebo, were increased blood creatine phosphokinase (placebo: 0.2% [1/401]; RBX: 2.2% [9/412]; $P = 0.012$), diabetic nephropathy (placebo: 0.2% [1/401]; RBX: 2.2% [9/412]; $P = 0.012$), tachycardia (placebo: 0.2% [1/401]; RBX: 1.7% [7/412]; $P = 0.036$); micturition urgency (placebo: 0.0% [0/401]; RBX: 1.2% [5/412]; $P = 0.027$); skin discoloration (placebo: 0.0% [0/401];

Table 3. Probable Causes of Vision Loss in Study Eyes of Patients Experiencing SMVL

Probable Causes of Vision Loss	Studies Combined		PKC-DRS		PKC-DRS2	
	Placebo SMVL Events (N = 48)	RBX SMVL Events (N = 26)	Placebo SMVL Events (N = 12)	RBX SMVL Events (N = 6)	Placebo SMVL Events (N = 36)	RBX SMVL Events (N = 20)
Center-involved DME, n (%)	38 (79.2)	17 (65.4)	12 (100.0)	5 (83.3)	26 (72.2)	12 (60.0)
Center-involved DME and cataract, n (%)	—	2 (7.7)	—	—	—	2 (10.0)
Cataract, n (%)	1 (2.1)	2 (7.7)	—	—	1 (2.8)	2 (10.0)
Vitreous hemorrhage, n (%)	1 (2.1)	4 (15.4)	—	1 (16.7)	1 (2.8)	3 (15.0)
Severe PDR, n (%)	2 (4.2)	—	—	—	2 (5.6)	—
Vascular occlusion, n (%)	4 (8.3)	1 (3.8)	—	—	4 (11.1)	1 (5.0)
Undetermined, n (%)	2 (4.2)	—	—	—	2 (5.6)	—

—, pathology did not account for any cases of SMVL in the given group.
PDR, proliferative DR.

RBX: 1.2% [5/412]; $P = 0.027$); papular rash (placebo: 0.0% [0/401]; RBX: 1.0% [4/412]; $P = 0.048$); retinal disorder (placebo: 0.0% [0/401]; RBX: 1.0% [4/412]; $P = 0.048$); and facial palsy (placebo: 0.0% [0/401]; RBX: 1.0% [4/412]; $P = 0.048$) (chi-square test or Fisher exact test). Despite increased blood creatine phosphokinaseand diabetic nephropathy being reported significantly more often in RBX-treated patients, there were no between-group differences in change in estimated glomerular filtration rate from baseline to endpoint or in the occurrence of other renal or diabetic microvascular complication–related adverse events (data not shown).

Discussion

These data demonstrate a beneficial effect of the isoform-selective PKC β inhibitor RBX in analyses of 813 patients (1,392 eyes) with moderately severe to very severe NPDR studied for 3 years with regard to SMVL, need for initial FPC, and visual gain. These combined analyses of the similarly designed PKC-DRS and PKC-DRS2 are consistent with earlier individual study findings,[10,11] although differences appear more statistically significant. While the absolute reduction in SMVL at 3 years in this combined analysis is relatively

Table 4. Adverse Events and Reasons for Discontinuation

Adverse Events	Studies Combined		PKC-DRS		PKC-DRS2	
	Placebo Patients (N = 401)	RBX Patients (N = 412)	Placebo Patients (N = 61)	RBX Patients (N = 67)	Placebo Patients (N = 340)	RBX Patients (N = 345)
Patients with ≥1 SAE, n (%)	178 (44.4)	168 (40.8)	21 (34.4)	23 (34.3)	157 (46.2)	145 (42.0)
Patients with ≥1 TEAE, n (%)	377 (94.0)	380 (92.2)	57 (93.4)	63 (94.0)	320 (94.1)	317 (91.9)
Reason for discontinuation						
Personal conflict/patient decision, n (%)	41 (10.2)	42 (10.2)	10 (16.4)	9 (13.4)	31 (9.1)	33 (9.6)
Unable to contact patient, n (%)	20 (5.0)	28 (6.8)	1 (1.6)	4 (6.0)	19 (5.6)	24 (7.0)
Adverse event, n (%)	11 (2.7)	19 (4.6)	2 (3.3)	3 (4.5)	9 (2.6)	16 (4.6)
Death, n (%)	23 (5.7)	18 (4.4)	1 (1.6)	4 (6.0)	22 (6.5)	14 (4.1)
Physician decision, n (%)	3 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.9)	0 (0.0)

There were no significant between-group differences for any reason for discontinuation, in either the studies combined or in the individual studies.
SAE, serious adverse event; TEAE, treatment-emergent adverse event.

modest because of low rates of SMVL overall (10.2 − 6.1% = 4.1%), 6-year long-term follow-up in a subset of the PKC-DRS2 patients revealed a more substantial absolute reduction in SMVL in patients treated with RBX for approximately 5 years of the follow-up period compared with those only receiving RBX for the final 2 years (26 − 8% = 18%).[19]

The integration of data from similarly designed studies is an acceptable practice that increases statistical power because of larger overall patient numbers and that can provide further confirmation of observed treatment effects when such effects are consistent among studies. For the PKC-DRS and PKC-DRS2, combination of data was appropriate because of similarities in patient demographics and the design and conduct of the 2 studies. The PKC-DRS was substantially smaller than the PKC-DRS2, and therefore would not be expected to greatly increase the power of the combined analyses. However, the similarity of the combined outcomes to those observed for the PKC-DRS and PKC-DRS2 individually and the more statistically significant results observed in the combined analyses demonstrate the consistency of the RBX treatment effects across the 2 studies.

The rate of SMVL was lower in the PKC-DRS2 than in the PKC-DRS (9% vs. 16% of placebo-treated patients, respectively). This difference may be explained in part by inclusion of patients with less severe DR (ETDRS level 47A in the worse eye) in the PKC-DRS2, but not the PKC-DRS. Patients with this milder level of DR represented nearly 40% of the PKC-DRS2 patient population, and the rate of SMVL in the PKC-DRS2 was lower in patients who had an ETDRS severity of 47A, as compared with those who had an ETDRS severity of 47B-D, in the worse eye (4.2% [5/119] vs. 5.9% [5/85] of placebo-treated patients, respectively). Additional factors that may have accounted for the remaining difference include better glycemic control at baseline in the PKC-DRS2 (HbA1c of 8.1% vs. 8.7%, on average), lower mean blood pressures at baseline in the PKC-DRS2 (systolic, 137.1 vs. 139.9 mmHg; diastolic, 77.4 vs. 80.6 mmHg), and improvements in the standard of care between 1998 to 1999 (when the PKC-DRS was enrolled) and 2001 to 2002 (when the PKC-DRS2 was enrolled).

Using stereo fundus photographs obtained every 6 months, we investigated the probable causes of vision loss in study eyes of patients who experienced SMVL. Center-involved DME was the predominant presumed cause of SMVL in all analyses, accounting for 60% to 100% of study eyes in each group.

Other probable causes of vision loss included cataract, vitreous hemorrhage, severe proliferative DR, and vascular occlusion. None of these other etiologies accounted for substantial numbers of study eyes in any group. Although a positive effect of RBX on DME was not observed in the PKC-DRS,[10] possibly because of the limited sample size, the current data, considered along with data presented in the primary publication of the PKC-DRS2,[11] would suggest that the impact of RBX on vision loss might be predominantly because of decreased progression of DME to the vision-threatening stage. A recent analysis of the effect of RBX on the relationship between visual acuity decline and duration of severe DME suggests that RBX may also provide some protection against the harmful effects of severe retinal thickening independent of its direct effects on DME.[20]

These data substantiate the effects of RBX in 2 independent studies evaluating 1,392 eyes in total. They suggest that RBX benefit to vision may be predominantly mediated by an effect on macular edema. Ruboxistaurin reduced vision loss over 3 years in patients with moderately severe to very severe NPDR, reduced the need for initial application of FPC, and increased the chance of visual acuity improvement. In addition, these data demonstrate that, consistent with previous studies[9–11,16–18] in patients with diabetic microvascular complications, RBX appears safe and well tolerated in patients with this severity of DR.

Key words: clinical trial, diabetes, diabetic retinopathy, PKC-DRS, PKC-DRS2, protein kinase C β, ruboxistaurin, vision loss.

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References

1. National Institute of Diabetes and Digestive and Kidney Diseases. National Diabetes Statistics Fact Sheet: General Information and National Estimates on Diabetes in the United States, 2005. Bethesda, MD: U.S. Department of Health and Human Services, National Institute of Health; 2005.
2. Way KJ, Katai N, King GL. Protein kinase C and the development of diabetic vascular complications. Diabet Med 2001;18:945–959.
3. Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. JAMA 2002;288:2579–2588.

4. Setter SM, Campbell RK, Cahoon CJ. Biochemical pathways for microvascular complications of diabetes mellitus. Ann Pharmacother 2003;37:1858–1866.
5. 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 beta-isoform-selective inhibitor. Diabetes 1997;46:1473–1480.
6. Danis RP, Bingaman DP, Jirousek M, Yang Y. Inhibition of intraocular neovascularization caused by retinal ischemia in pigs by PKC beta inhibition with LY333531. Invest Ophthalmol Vis Sci 1998;39:171–179.
7. Nonaka A, Kiryu J, Tsujikawa A, et al. PKC beta inhibitor (LY333531) attenuates leukocyte entrapment in retinal microcirculation of diabetic rats. Invest Ophthalmol Vis Sci 2000;41:2702–2706.
8. Yokota T, Ma RC, Park JY, et al. Role of protein kinase C on the expression of platelet-derived growth factor and endothelin-1 in the retina of diabetic rats and cultured retinal capillary pericytes. Diabetes 2003;52:838–845.
9. Aiello LP, Clermont A, Arora V, et al. Inhibition of PKC beta by oral administration of ruboxistaurin is well tolerated and ameliorates diabetes-induced retinal hemodynamic abnormalities in patients. Invest Ophthalmol Vis Sci 2006;47:86–92.
10. 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 beta Inhibitor Diabetic Retinopathy Study (PKC-DRS) multicenter, randomized clinical trial. Diabetes 2005;54:2188–2197.
11. The PKC-DRS2 Study Group. The effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology 2006;113:2221–2230.
12. 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.
13. Ophthalmology AAO.International clinical classification system for diabetic retinopathy and diabetic macular edema. http://www.aao.org/education/library/recommendations/international_dr.cfm. Accessed April 6, 2006.
14. Early Treatment Diabetic Retinopathy Study Research Group.-Early Treatment Diabetic Retinopathy Study (ETDRS) Manual of Operations. Springfield, VA: U.S. Department of Commerce, National Technical Information Service; 1993.
15. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology 1991;98:786–806.
16. The PKC-DMES Study Group. Effect of ruboxistaurin, a PKC β isoform-selective inhibitor, in patients with diabetic macular edema: 30-month results of the randomized PKC-DMES clinical trial. Arch Ophthalmol 2007;125: 318–324.
17. Tuttle KR, Bakris GL, Toto RD, et al. The effect of ruboxistaurin on nephropathy in type 2 diabetes. Diabetes Care 2005;28:2686–2690.
18. McGill JB, King GL, Berg PH, et al. Clinical safety of the selective PKC-beta inhibitor, ruboxistaurin. Expert Opin Drug Saf 2006;5:835–845.
19. Sheetz MJ, Aiello LP, Shahri N, Davis MD, Kles KA, Danis RP; for the MBDV Study Group. Effect of Ruboxistaurin (RBX) on Visual Acuity Decline Over a 6-Year Period With Cessation and Reinstitution of Therapy - Results of an Open-Label Extension of the Protein Kinase C Diabetic Retinopathy Study 2 (PKC-DRS2). Retina. 2011 March 3. [epub ahead of print].
20. Davis MD, Sheetz MJ, Aiello LP, et al; PKC-DRS2 Study Group. Effect of ruboxistaurin on the visual acuity decline associated with long-standing diabetic macular edema. Invest Ophthalmol Vis Sci 2009;50:1–4.

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