A 6-Month, Randomized, Double-Masked, Placebo-Controlled Study Evaluating the Effects of the Protein Kinase C-β Inhibitor Ruboxistaurin on Skin Microvascular Blood Flow and Other Measures of Diabetic Peripheral Neuropathy

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OBJECTIVE — Diabetes leads to protein kinase C (PKC)-β overactivation and microvascular dysfunction, possibly resulting in disordered skin microvascular blood flow (SkBF) and other changes observed in diabetic peripheral neuropathy (DPN) patients. We investigate the effects of the isoform-selective PKC-β inhibitor ruboxistaurin mesylate on neurovascular function and other measures of DPN.

RESEARCH DESIGN AND METHODS — Endothelium-dependent and C fiber–mediated SkBF, sensory symptoms, neurological deficits, nerve fiber morphometry, quantitative sensory and autonomic function testing, nerve conduction studies, quality of life (using the Norfolk Quality-of-Life Questionnaire for Diabetic Neuropathy [QOL-DN]), and adverse events were evaluated for 20 placebo- and 20 ruboxistaurin-treated (32 mg/day) DPN patients (aged ≥18 years; with type 1 or type 2 diabetes and A1C ≤11%) during a randomized, double-masked, single-site, 6-month study.

RESULTS — Endothelium-dependent (+78.2%, $P < 0.03$) and C fiber–mediated (+56.4%, $P < 0.03$) SkBF at the distal calf increased from baseline to end point. Significant improvements from baseline within the ruboxistaurin group were also observed for the Neuropathy Total Symptom Score-6 (NTSS-6) (3 months −48.3%, $P = 0.01$; end point −66.0%, $P < 0.0006$) and the Norfolk QOL-DN symptom subscore and total score (end point −41.2%, $P = 0.01$, and −41.0, $P = 0.04$, respectively). Between-group differences in baseline–to–end point change were observed for NTSS-6 total score (placebo −13.1%; ruboxistaurin −66.0%, $P < 0.03$) and the Norfolk QOL-DN symptom subscore (placebo −4.0%; ruboxistaurin −41.2%, $P = 0.041$). No significant ruboxistaurin effects were demonstrated for the remaining efficacy measures. Adverse events were consistent with those observed in previous ruboxistaurin studies.

CONCLUSIONS — In this cohort of DPN patients, ruboxistaurin enhanced SkBF at the distal calf, reduced sensory symptoms (NTSS-6), improved measures of Norfolk QOL-DN, and was well tolerated.

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Diabetic peripheral neuropathy (DPN) is often characterized by damage to both large myelinated Aα and Aβ nerve fibers, as well as small, thinly myelinated C fibers (1–4). Small-fiber damage may occur first in the lower limb and may precede large-fiber damage, making it one of the earliest indicators of the onset of DPN (1,4).

Small-fiber DPN is associated with increased morbidity and mortality. Symptoms include numbness, pain, and decreased sensation, as well as autonomic symptoms such as anhidrotic skin, orthostatic hypotension, resting tachycardia, hypoglycemia unawareness, delayed gastric emptying, decreased bladder tone, and impotence (1–3,5).

In small-fiber DPN, numbness, decreased sensation, and anhidrotic skin with disordered microvascular blood flow conspire to predispose patients to foot ulcers and amputation. The presence and duration of diabetes are strong predictors of lower extremity amputation risk (6). In the U.S., diabetes complications are the leading cause of nontraumatic amputations, accounting for 86,000 per year (7), or 1 every 10 min. Treatments to improve microvascular dysfunction in DPN patients could improve their prognosis.

Protein kinase C (PKC)-β overactivation has been associated with DPN (8–10). PKC-β is overactivated by hyperglycemia and by disordered fatty acid metabolism, resulting in increased production of vasoconstrictive, angiogenic, and chemotactic cytokines, including transforming growth factor-β, vascular endothelial growth

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Abbreviations: DPN, diabetic peripheral neuropathy; NIS, Neurologic Impairment Score; NSS, Neuropathy Symptom Score; NTSS-6, Neuropathy Total Symptom Score-6; PKC, protein kinase C; QOL-DN, Quality-of-Life Questionnaire for Diabetic Neuropathy; QST, quantitative sensory testing; SAE, serious adverse event; SkBF, skin microvascular blood flow; TEAE, treatment-emergent adverse event; VDT, vibration detection threshold.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
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factor, endothelin-1, and intercellular adhesion molecules (11).

Ruboxistaurin mesylate specifically inhibits PKC-β overactivation, blocking a critical step in the pathogenesis of DPN via its impact on a microvascular mechanism (8). A 1-year, multinational, randomized, phase 2, double-masked, placebo-controlled study (MBBQ) evaluated ruboxistaurin's efficacy and safety in DPN patients. Although the study failed to achieve the primary end point of improvement in quantitative sensory testing (QST) for vibration detection threshold (VDT), it demonstrated that ruboxistaurin reduced sensory symptoms and improved VDT in a subset of patients with mild DPN (12).

There are few data regarding ruboxistaurin's effect on skin microvascular blood flow (SkBF). Therefore, we conducted a 6-month, randomized, double-masked, placebo-controlled study in DPN patients, evaluating ruboxistaurin's effect on endothelium-dependent and C fiber–mediated SkBF (primary end points) and on secondary end points of sensory symptoms, neurological deficits, nerve fiber morphometry, QST, quantitative autonomic function testing, nerve conduction studies, quality of life, and adverse events. We hypothesized that ruboxistaurin would primarily increase SkBF and secondarily improve QST and nerve fiber morphometry.

RESEARCH DESIGN AND METHODS

— Patients had type 1 or type 2 diabetes with bilateral sensorimotor distal peripheral neuropathy attributable to diabetes, with an abnormal test in two or more of the following measures: Neuropathy Total Symptom Score-6 (NTSS-6) ≥6 points (total score) or ≥2.33 points for one or more symptom (13); VDT ≥75th percentile for age, sex, and anthropometric measures (14); warm thermal perception threshold ≥12.4°C; cold thermal perception threshold ≥10.5°C; age-corrected abnormality of cardiovascular reflex testing of autonomic function (heart rate variability during deep breathing [expiration-to-inspiration or E:I ratio] or R:R variation in response to the Valsalva maneuver or postural change); and evidence of distal symmetric polyneuropathy based on peroneal motor nerve conduction studies, as measured by an abnormality in one of the following: amplitude ≤4 mV, distal latency ≥5 ms, or conduction velocity ≤40 m/s. Patients had A1C ≤11% and were aged ≥18 years.

Exclusion criteria included neurological disease or neuropathy from a cause other than diabetes, advanced neurological disease (either peroneal nerve conduction velocity <30 m/s or VDT >23 just-noticeable difference units), uncontrolled diabetes (more than two episodes of ketoacidosis or hyperosmolar state requiring hospitalization and/or six or more episodes of hypoglycemia requiring assistance within 3 months before study entry), blood pressure ≥160/95 mmHg, impaired renal function or active hepatic disease, pregnancy or lactation, and suspected carcinoma or history of carcinoma within 5 years before study entry.

This was a 6-month, randomized, placebo-controlled, double-masked, single-site study with a within- and between-patient design. SkBF, sensory symptoms, and adverse events were evaluated at baseline, 3 months, and 6 months (end point). The remaining measures were evaluated at baseline and end point.

Patients were randomized to placebo ($n = 20$), 32 mg ruboxistaurin ($n = 20$), or 64 mg ruboxistaurin ($n = 12$) orally once per day for 6 months. Sample size was based on previous SkBF data generated using the same methodologies described herein (2,15), which demonstrated that 40 patients (20 per arm) needed to be randomized to achieve ≥80% power for observing statistical significance at the $P = 0.05$ level for the 32 mg/day ruboxistaurin group. Because the 64 mg/day ruboxistaurin group was underpowered and was included only as a pilot arm, we report data for only the primary comparison between the two adequately powered arms, placebo and 32 mg/day ruboxistaurin.

The study was performed in accordance with the principles of the Declaration of Helsinki and was reviewed and approved by the Eastern Virginia Medical School Institutional Review Board. Patients gave written informed consent before any study-related procedure.

Efficacy measurements

SkBF was noninvasively measured at two sites over the external aspect of the nondominant leg by laser Doppler, using Periflux small-angled thermostatic probes (Perimed, North Royalton, OH) with core diameters of 0.125 mm, laser wavelengths of 780 nm, and fiber separation lengths of 0.25 mm. This system measures skin perfusion as erythrocyte flux. The depth of the measurements, influenced by tissue properties, light source, and probe configuration, was estimated to be 500–1,000 μm.

The methods used to measure SkBF have been previously described (15–17). Briefly, laser Doppler probes were positioned 10 cm below the knee and 10 cm above the malleolus in a thermoneutral (25°C) laboratory environment. SkBF (measured in perfusion units) was first assessed at basal body temperature for 10 min and then reassessed as the skin was heated to 32°C for 10 min, 40°C for 10 min (eliciting endothelium-dependent vasodilation) (18), and 44°C for 40 min (eliciting C fiber–mediated vasodilation) (18). Percent increases in SkBF from basal temperature to 40°C and 44°C were calculated at baseline and after 3 and 6 months.

Sensory symptoms were measured using two questionnaires: the NTSS-6, which quantifies frequency and intensity of aching, burning, prickling and lancinating pain, numbness, and allodynia in patients' feet and legs (13), and the Neuropathy Symptom Score (NSS), which quantifies symptoms of motor, sensory, and autonomic deficits (19).

Neurological deficits were measured using the Neurologic Impairment Score (NIS), composed of a sensory subscore (which evaluates sensory perceptions to touch, prickling pain, vibration, joint position, and 1- and 10-g monofilaments in the upper and lower extremities) and a motor subscore (which evaluates cranial nerves, muscle strength, muscle wasting, and deep tendon reflexes in the upper and lower extremities) (modified from ref. 14).

Skin punch biopsies (3 mm) were taken at the same two sites indicated in the SkBF studies on the nondominant limb. Patients were pretreated with 2% lidocaine. Tissue samples were immediately fixed in 2% buffered paraformaldehyde/lysine/periodate solution for 12–24 h at 4°C, cryoprotected in phosphate buffer with 20% glycerol, and frozen for later cryosectioning.

Immunofluorescence was used to identify neurons in skin biopsies, using a protocol modified from that of McCarthy et al. (20). Specimens were cut in 50-μm sections on a Reichert cryostat. Melanin was bleached with a 0.25% $KMnO_4$ solution, followed by a 5% oxalic acid solution. Tissues were permeabilized and blocked with a solution of Tris buffer with 0.5% powdered milk, 1.0% Triton X-100, and 4% normal goat serum. Sections were stained in the same solution with rabbit

anti-PGP (protein gene product) 9.5 antibody (Chemicon, Temecula, CA) overnight at 4°C and incubated with fluorescein isothiocyanate–labeled goat anti-rabbit IgG secondary antibody (Sigma, St. Louis, MO) for 2 h. After washing, slides were covered with Vectashield and sealed with nail polish.

Images were captured by a masked observer using an Olympus Fluoview Scanning Laser Confocal Microscope. Six random nonoverlapping fields from at least three different sections of each specimen were collected for analysis. Confocal images were captured at 1.6-μm intervals, and image stacks were superimposed to produce the image for quantification. Quantification was performed only on the area of the photomicrographs showing epidermis. Images were analyzed for mean dendritic length (μm), dendrite number, and intraepidermal nerve fiber density (mm epidermis^{-1}) using Image Pro Plus software (Media Cybernetics, Silver Spring, MD).

QST was performed using the Medoc device according to previously published methods (16). Patients were tested for warm thermal and cold thermal thresholds and heat and cold pain using a computer-driven sensory measuring device (TSA2001/VSA3001; Medoc, Durham, NC). Touch pressure was measured using graded Semmes-Weinstein monofilaments (21). Measurements were taken 2 cm proximal to the sites of skin biopsies, on the nondominant limb. VDT was performed on the nondominant foot using the Computer-Aided Sensory Evaluator IV device, according to previously published methods and algorithms (22–26).

Autonomic function was assessed as suggested by the American Diabetes Association position statement on neuropathy (27), by three tests using an ANSAR device: heart rate variability during deep breathing at six breaths per min (E:I ratio) and R:R variation in response to both the Valsalva maneuver and postural change.

Surface stimulation and recordings of nerve conduction were obtained from the sural, peroneal, and tibial nerves of the nondominant lower extremity. Amplitude, distal latency of compound muscle action potentials and sensory nerve action potentials, and motor nerve F-wave latency were measured, and conduction velocities were calculated using standard methods (28–29).

Patients completed the self-administered Norfolk Quality-of-Life Questionnaire for Diabetic Neuropathy (QOL-DN), which has been shown to provide good to excellent correlations between symptomatic DPN and quality of life from the patient's perspective (30). The Norfolk QOL-DN is composed of questions related to patients' signs and symptoms, as well as to the impact of DPN on patients' activities of daily life.

Adverse event documentation

Adverse events and their relationship to the study drug were recorded at each visit. Treatment-emergent adverse events (TEAEs) were those that were not present before initiation of the study drug or those that worsened either in intensity or frequency following study drug exposure. Serious adverse events (SAEs) were those that resulted in death, hospitalization, life-threatening consequences, severe or permanent disability, cancer, or other significant consequence.

Statistical analyses

The study design was parallel, with a within- and between-patients repeated-measures ANOVA analysis. The primary dependent variable was SkBF, and secondary dependent variables were QST and indexes of nerve fiber morphometry, all of which are continuous data. Independent variables were the treatment groups (two levels). For within- and between-group comparisons of demographic and laboratory data, nonparametric tests (Wilcoxon's signed-rank test and Mann-Whitney *U* test, respectively) were used. Nonparametric tests were used to examine within- and between-group treatment effects for all efficacy measures. All tests were two sided. $P < 0.05$ was significant. Where significant treatment differences were observed, contrast testing was used to determine the significance at each level, while allowing for multiplicity of planned comparisons.

RESULTS

Baseline characteristics

There were no between-group differences with regard to baseline characteristics, except for percent of patients receiving insulin (placebo 75%; ruboxistaurin 38%, $P < 0.004$) and motor subscore (placebo median 3.8 points [interquartile range 1.6–4.8]; ruboxistaurin 1.2 points [1.0–2.4], $P = 0.0007$) (Table 1).

Efficacy

Effects of ruboxistaurin on SkBF at the distal calf are shown in Fig. 1*A* and *B*. At end point, increases from baseline in SkBF at the distal calf were significant in the ruboxistaurin group but not the placebo group (endothelium-dependent response: placebo +22.5%, P = NS; ruboxistaurin +78.2%, $P < 0.03$; C fiber–mediated response: placebo +65.9%, $P = 0.07$; ruboxistaurin +56.4%, $P < 0.03$). There were no significant baseline–to–3 month changes in endothelium-dependent or C fiber–mediated SkBF within groups and no significant between-group differences in change from baseline in endothelium-dependent or C fiber–mediated SkBF after 3 or 6 months.

No significant within- or between-group ruboxistaurin effects were demonstrated for SkBF at the proximal calf after 3 or 6 months (6 months: endothelium-dependent response: placebo +26.5%, ruboxistaurin +25.0%, C fiber–mediated response: placebo +36.5%, ruboxistaurin +32.8% [all P = NS]).

After 3 and 6 months, NTSS-6 total score decreased significantly from baseline in the ruboxistaurin group (−48.3%, $P = 0.01$, and −66.0%, $P < 0.0006$, respectively) but not the placebo group (−18.9% and −13.0%, respectively, both P = NS) (Fig. 2). The baseline–to–end point decrease observed for the NTSS-6 total score was significantly greater in the ruboxistaurin group than the placebo group ($P < 0.03$) (Fig. 2).

NSS decreased from baseline to end point in both groups, but these within-group changes (placebo −13.8%, ruboxistaurin −20.8%) were nonsignificant. The between-group difference in NSS baseline–to–end point change was also nonsignificant.

NIS decreased from baseline to end point in both groups, but these within-group changes (placebo −26.6%, ruboxistaurin −24.3%) and the between-group difference in NIS baseline–to–end point change were not statistically different.

Sensory subscore (−29.8%) and motor subscore (−5.9%) were not significantly changed from baseline to end point within the ruboxistaurin group, while within the placebo group, motor subscore (−31.4%, $P = 0.03$), but not sensory subscore (−23.4%, P = NS), decreased significantly from baseline to end point. The baseline–to–end point change in motor subscore observed for the placebo group was borderline significantly greater than that observed for the ruboxistaurin group ($P < 0.07$), while the baseline–to–end

Table 1—*Characteristics of placebo- and ruboxistaurin-treated patients at baseline*

Characteristic	Placebo	32 mg/day ruboxistaurin	*P*
n	20	20	
Age (years)	56.7 (51.1–65.0)	61.5 (47.4–63.4)	NS
Male	17 (85)	15 (75)	NS
Female	3 (15)	5 (25)	
Caucasian origin	15 (75)	16 (80)	
African-American origin	5 (25)	2 (10)	NS
Other origin	0	2 (10)	
Type 1 diabetes	4 (20)	3 (15)	NS
Type 2 diabetes	16 (80)	17 (85)	
Duration of diabetes (years)	14.8 (8.9–22.2)	12.3 (3.8–16.8)	NS
Duration of DPN (years)	4.6 (0.9–9.5)	2.3 (0.1–11.1)	NS
Alcohol users	3 (15)	6 (30)	NS
Smokers	2 (10)	0 (0)	NS
BMI (kg/m^2)	29.4 (25.5–36.3)	29.3 (26.0–33.8)	NS
Waist (cm)	106.0 (94.0–125.0)	107.5 (93.3–119.3)	NS
A1C (%)	7.8 (6.6–8.6)	6.8 (6.2–7.9)	NS
C-peptide (ng/ml)	2.0 (0.5–2.6)	2.1 (1.7–2.7)	NS
Total cholesterol (mg/dl)	160 (143–204)	175 (153–203)	NS
Triglycerides (mg/dl)	96 (67–141)	73 (55–180)	NS
HDL cholesterol (mg/dl)	45.8 (40.3–59.0)	50.1 (43.8–64.0)	NS
LDL cholesterol (mg/dl)	94 (77–118)	91 (68–128)	NS
SBP (mmHg)	130.3 (126.9–138.8)	132.8 (120.4–145.4)	NS
DBP (mmHg)	76.8 (65.9–81.3)	74.2 (68.4–78.3)	NS
Insulin treatment	15 (75)	6 (30)	0.004
NTSS-6 total score (points)	3.3 (0.4–9.0)	4.6 (2.0–6.8)	NS
NSS (points)	5.0 (3.6–8.0)	4.0 (3.0–7.0)	NS
NIS (points)	9.5 (4.6–15.5)	6.5 (4.0–10.0)	NS
Sensory score (points)	5.8 (2.6–12.0)	4.8 (3.0–8.4)	NS
Motor score (points)	3.8 (1.6–4.8)	1.2 (1.0–2.4)	0.0007

Data are *n* (%) or median (interquartile range). *P* value denotes between-group comparisons. DBP, diastolic blood pressure; NS, not significant; SBP, systolic blood pressure.

point change in sensory subscore was not significantly different between groups.

A significant between-group difference in baseline–to–end point change in the Norfolk QOL-DN symptom subscore was observed (placebo −4.0%; ruboxistaurin −41.2%, $P = 0.041$). Also, a significant within-group difference for symptom subscore (−41.2%, $P = 0.01$) and total score (−41.0%, $P = 0.04$) was observed for the ruboxistaurin group, but not the placebo group, after 6 months. No other significant within-group changes from baseline or between-group differences in change from baseline were demonstrated for the remaining Norfolk QOL-DN measures.

No significant within- or between-group ruboxistaurin effects were observed for the remaining efficacy measures (nerve fiber morphometry, QST, quantitative autonomic function testing, and nerve conduction studies) (supplemental appendix I [available in an online appendix at http://dx.doi.org/10.2337/dc06-1699]).

Safety profile

Thirty-five of 40 (87.5%) patients experienced one or more TEAE during the study. TEAEs occurring in more than two patients (>5% frequency) included shoulder pain (placebo 1, ruboxistaurin 3), Dupuytren contracture (placebo 1, ruboxistaurin 2), upper respiratory infection (placebo 1, ruboxistaurin 3), left atrial hypertrophy (placebo 1, ruboxistaurin 3), skin biopsy site reaction (placebo 6, ruboxistaurin 3), cough (placebo 2, ruboxistaurin 3), and premature ventricular contraction (placebo 2, ruboxistaurin 1).

Four SAEs were reported in the 32 mg/day ruboxistaurin group: bacterial pneumonia, myocardial ischemia, coronary artery disease with stent placement, and death resulting from acute myocardial infarction. No SAEs were considered by the principal investigator to be related to the study drug, except for myocardial ischemia, for which a causal relationship with ruboxistaurin could not be ruled out. No patient discontinued the study because of an adverse event, except for the patient who died due to an acute myocardial infarction.

CONCLUSIONS — This randomized, double-masked, placebo-controlled, single-site study, which evaluated 40 DPN patients treated with either placebo or 32 mg/day ruboxistaurin for 6 months, demonstrated that ruboxistaurin had positive within-group treatment effects on endothelium-dependent and C fiber–mediated SkBF at the distal calf, sensory symptoms (NTSS-6), and measures of the QOL-DN. Ruboxistaurin's effect on endothelium-dependent SkBF appeared to be more robust than that on C fiber–mediated SkBF because ruboxistaurin improved endothelium-dependent SkBF ~50% more than placebo, whereas baseline–to–end point increases in C fiber–mediated SkBF were similar between groups (placebo +65.9%, ruboxistaurin +56.4%). Ruboxistaurin had positive between-group treatment effects on change from baseline in the NTSS-6 total score and Norfolk QOL-DN symptom subscore. No significant within- or between-group effects of ruboxistaurin were demonstrated for the remaining efficacy measures. TEAEs were similar between groups, and the adverse event profile was consistent with that previously reported for ruboxistaurin (12,31–35).

The objective of this study was to explore the role of PKC-β in the pathogenesis of vascular and neural complications of diabetes. PKC plays a central role in cellular regeneration and has at least 12 isoforms important in signal transduction (36). The β isoform is preferentially overactivated in a variety of vascular tissues during hyperglycemia (8–10). Specific inhibition of PKC-β by ruboxistaurin has been shown to normalize changes in microvascular function, including retinal blood flow (37), endoneurial blood flow (38–39), and sensory and motor nerve conduction velocities (38–40), in animal models of diabetes. Moreover, specific inhibition of PKC-β by ruboxistaurin has been shown to ameliorate diabetes-induced retinal hemodynamic abnormalities in diabetic patients (33).

MBBQ was a multinational, randomized, phase 2, double-masked, placebo-controlled study evaluating ruboxistaurin's efficacy and safety over 1 year in DPN patients (12). Although the study failed to achieve the primary end point of improvement in QST for VDT, it demonstrated that ruboxistaurin significantly

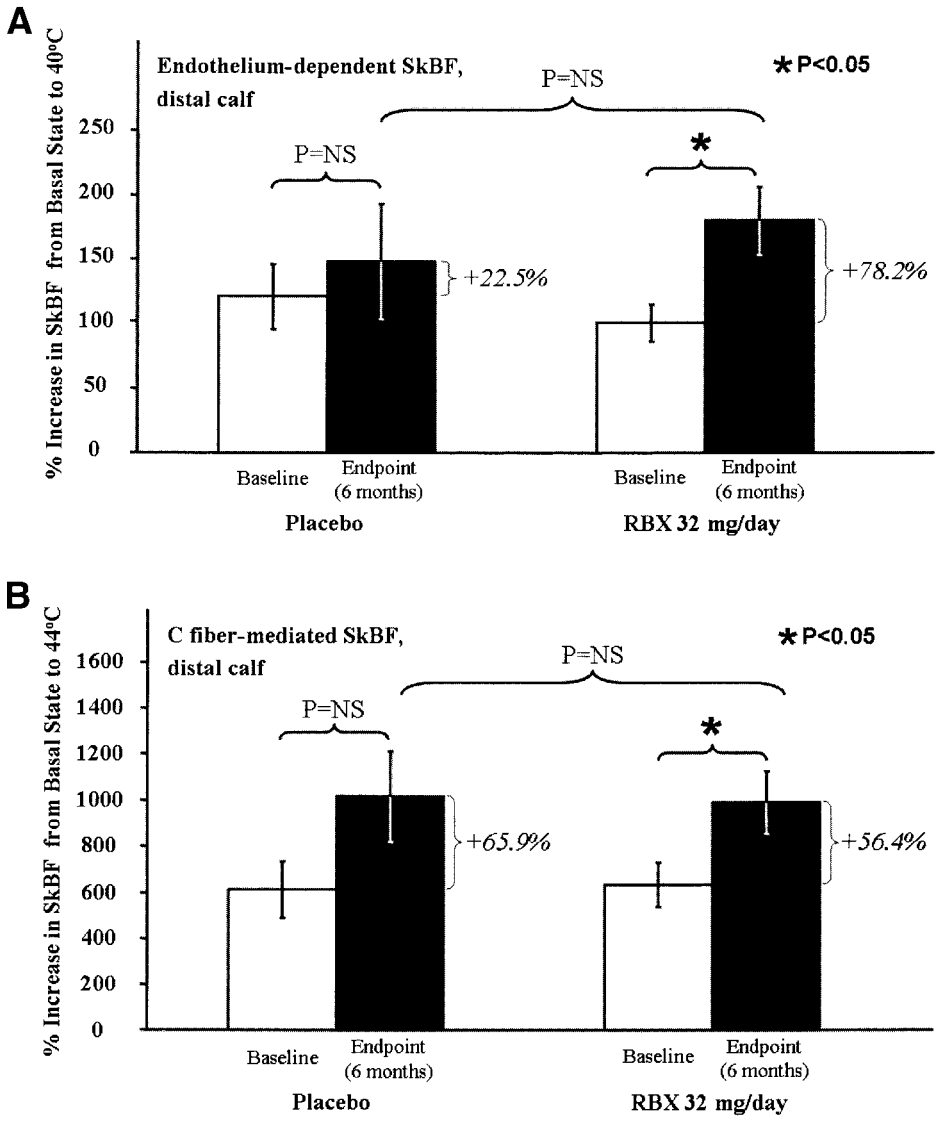

Figure 1—A: *Percent increase in SkBF resulting from heating the skin from basal temperature to 40°C (and thereby eliciting endothelium-dependent vasodilation) at the distal calf, measured at baseline (□) and after 6 months (■) in placebo- and ruboxistaurin (RBX)-treated patients.* B: *Percent increase in SkBF resulting from heating the skin from basal temperature to 44°C (and thereby eliciting C fiber–mediated vasodilation) at the distal calf, measured at baseline (□) and after 6 months (■) in placebo- and ruboxistaurin-treated patients. For each treatment group, the percent difference in these changes from basal state, measured from baseline to end point (6 months), is shown in italic font. No significant within- or between-group ruboxistaurin effects were observed after 3 months of treatment.*

reduced sensory symptoms of DPN compared with placebo (12). Moreover, in a subset of patients with intact sural nerve amplitudes, ruboxistaurin reduced sensory symptoms and improved VDT. SkBF and nerve fiber morphometry were not evaluated in this study, and the population characteristics differed from the current study, in that MBBQ patients had a longer duration of diabetes, more cases of type 1 diabetes, and a higher A1C value at baseline.

In the current study, we observed a significant decrease from baseline in motor subscore in the placebo, but not the ruboxistaurin, group. The greater motor subscore observed in the placebo group at baseline (Table 1) likely contributed to a regression to the mean. We also observed a placebo effect with regard to changes from baseline in C fiber–mediated SkBF and NIS. The observance of a placebo effect is consistent with previous short-duration studies in DPN patients, most likely due to the variability in the responses measured. Long-term study of nerve function is warranted and necessary to detect consistent changes in neuropathy deterioration. Placebo effects may be related to the universal use of statins and ACE inhibitors and to an awareness of the need for increased exercise and proper nutrition; however, the exact reason for such effects remains obscure (41).

Data regarding the effect of drugs used for the treatment of DPN symptoms on changes in SkBF are scarce. This is one of the first studies to evaluate the effect of a drug being investigated for the treatment of DPN (ruboxistaurin) on SkBF in diabetic patients. Recently, we showed that 12 weeks of treatment with topiramate increased SkBF in DPN patients. SkBF returned to baseline and was followed by significant increases in intraepidermal nerve fiber density and dendritic length, accompanied by changes in small-fiber sensory perception, by 18 weeks

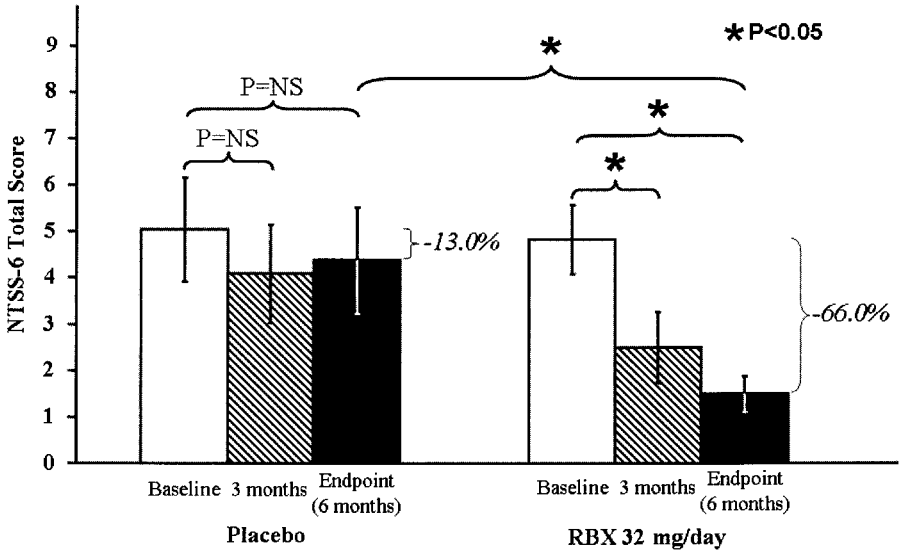

Figure 2—*NTSS-6 total score at baseline (□) and after 3 (▨) and 6 (■) months in placebo- and ruboxistaurin (RBX)-treated patients. For each treatment group, the percent difference in NTSS-6 total score, measured from baseline to end point (6 months), is shown in italic font.*

(42). This finding suggests that there is a scope for combination therapies for improving SkBF using drugs that operate by different mechanisms of action.

Our study has limitations. Since the patient population was well defined at baseline, it is unclear whether the same ruboxistaurin effects would be observed after 6 months of treatment in the general population of DPN patients, including those with more severe disease. Also, since the study was only 6 months in duration, it is unclear what the effect of ruboxistaurin would be on SkBF and other measures of DPN at later time points. Studies of longer duration would be necessary to confirm that the observed changes occur and are sustained long term.

Data from this cohort of DPN patients suggest that treatment of patients with neuropathy with the isoform-specific PKC-β inhibitor ruboxistaurin may reduce sensory symptoms and improve endothelial-dependent skin blood flow. It has been hypothesized that changes in other measures of DPN may occur after longer periods of ruboxistaurin treatment; further placebo-controlled studies of longer duration would be necessary to confirm this hypothesis.

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