Progression of Diabetic Macular Edema: Correlation with Blood–Retinal Barrier Permeability, Retinal Thickness, and Retinal Vessel Diameter

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Purpose. To study the progression of diabetic macular edema (DME) in relation to baseline retinal thickness, retinal vascular leakage, and retinal trunk vessel diameters.

Methods. In this single-center study, 45 patients were enrolled with 62 eligible eyes defined as having DME of a grade less than clinically significant macular edema (CSME). From the start, the patients were included in a multicenter study exploring the effect of ruboxistaurin versus placebo for 3.4 years. Subsequently, the patients were followed up for a mean of 5.7 years by optical coherence tomography, fundus photography, and vitreous fluorometry. Baseline values in eyes that progressed to photocoagulation treatment were compared with values from eyes that did not reach this endpoint.

Results. In the 22 eyes of 18 patients in which CSME was diagnosed and treated, mean retinal vascular leakage at baseline was 5.6 (95% CI 4.2–7.6) nm/s, whereas eyes that did not progress to photocoagulation had a significantly lower mean leakage at baseline of 3.4 (95% CI 2.7–4.3) nm/s. No significant difference was found for measures of baseline retinal thickness or summarized retinal trunk vessel diameters. Eyes that progressed to photocoagulation treatment (mean delay to treatment, 3.6 years) had significantly higher foveal thicknesses than did nonprogressing eyes, from 18 months after study initiation.

Conclusions. Progression to photocoagulation treatment for CSME was associated with higher retinal vascular leakage at baseline, whereas baseline retinal vessel diameters and retinal thickness were comparable in progressing and nonprogressing eyes. Baseline leakage was the strongest predictor of progression from non-CSME to photocoagulation for CSME. (*Invest Ophthalmol Vis Sci.* 2007;48:3983–3987) DOI:10.1167/iovs.06-1102

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Diabetic macular edema (DME) usually begins as smaller areas of edema that do not involve the fovea, from which it can progress to stages that threaten or manifestly involve the center of the macula. The Early Treatment of Diabetic Macular Edema (ETDRS) defined a threshold for photocoagulation treatment, clinically significantly diabetic macular edema (CSME) that has become an important guideline for intervention in clinical practice. Retinal thickness is usually evaluated by stereoscopic biomicroscopy or fundus photography.[1,2] Objective assessment of retinal thickness by optical coherence tomography (OCT) correlates with stereoscopic assessment of edema.[3] Mechanistically, the relation between retinal vascular leakage of fluorescein and retinal thickening is of interest, and vitreous fluorometry has demonstrated higher leakage in CSME than in lesser grades of DME.[4,5] Also of interest is the relation between changes in retinal vessel diameters and retinal thickening.[6,7] The temporal relation between the various aspects of dysfunction and thickening has not been described in detail, and it remains uncertain whether increased leakage precedes, accompanies, or follows retinal thickening. In the present study, we examined retinal fluorescein leakage and retinal thickness and summarized retinal trunk vessel diameters prospectively in patients with DME of less than CSME grade at baseline. The data evaluated in this study were acquired from a placebo-controlled study of pharmacologic protein kinase C βII inhibition using an orally administered PKC βII-inhibitor, ruboxistaurin mesylate (LY333531).[8,9]

Methods

Subjects

The study enrolled 74 eyes in 54 patients with DME in at least one eye in a randomized controlled multicenter trial of ruboxistaurin (4, 16, or 32 mg/d) versus placebo. Nine patients failed to complete the study: three patients died (one of cardiovascular disease, two of cancer), five patients withdrew their consent for reasons unrelated to the study. One patient suffered an adverse event (skin rash) and was withdrawn from the study. These withdrawals left 62 eyes in 45 patients for analysis. The study population comprised 15 women and 30 men, of whom 13 had type I and 32 had type II diabetes.

This study was a single-center extension of the multicenter trial. The patients were examined at 3-month intervals until the final visit of the study. The time to the final study visit was longer the earlier the date of enrollment and ranged from 2.8 to 4.3 years (mean, 3.4) depending on the time of inclusion. At the final visit, the study medicine was discontinued. Vitreous fluorometry, retinal thickness, and fundus photographic retinal vessel caliber measurements were performed at baseline, 12 and 18 months, and the final visit.

After the last study visit, all patients were examined clinically every 3 months until follow-up closed or, if macular edema was not found or clearly not approaching CSME grade, the patient was followed up at our fundus photographic screening unit at comparable intervals. All events of CSME were diagnosed by the same ophthalmologists. An overview of the examinations is given in Table 1.

Table 1. Outline of Scheduled Examinations in Study of Patients with Non-CSME at Baseline

	Baseline	1 Year	1.5 Years	Final Study Visit	First Poststudy Visit	Final Follow-up, Untreated Eyes
Interval (mean)	0	1	1.5	3.4	3.8	5.7
Range (y)	—	—	—	2.75–4.25	3.2–4.25	3.3–7.5
Types of tests	VA, BM, FP, VF/FA, OCT	VA, BM, FP, VF/FA, OCT	VA, BM, FP, VF/FA, OCT	VA, BM, FP, VF/FA, OCT	VA, BM, FP	VA, BM, FP, OCT

Protocolized visits were conducted regularly up to 1.5 years after baseline and up to the final study visit. Additional visits were conducted thereafter, to survey the incidence of photocoagulation for DME. The tests included: BM, biomicroscopy; VA, visual acuity; VF, vitreous fluorometry; FA, fluorescein angiography; OCT, retinal thickness measurement by optical coherence tomography; and FP, stereoscopic fundus photography.

Informed consent was obtained from all patients before they entered the study, which was conducted according to the ICH-Guidelines for Good Clinical Practice (ICH GCP) and the Declaration of Helsinki and was approved by the Danish Medicines Agency and regional medical ethics committee. Ocular eligibility was defined as presence of ≥¼ disc area (DA) of definite retinal thickening within 2 disc diameters (DD) of the center of the macula and ETDRS severity of retinopathy level ≤47A, as determined by stereoscopic fundus photograph grading.[2] Ocular ineligibility was defined as presence of CSME, except that retinal thickening or hard exudates adjacent to retinal thickening at/or within 500 μm but no closer than 300 μm from the fovea was allowed if visual acuity was ≥75 ETDRS letters.[8]

Study Design

The present study is an exploratory investigation of blood–retinal barrier permeability, retinal thickness, and retinal vessel measurements to elucidate the development of macular edema. The advent of CSME was defined as DME severe enough to necessitate macular photocoagulation, as determined by clinical evaluation based on ETDRS criteria.

Retinopathy

The level of retinopathy was graded on seven-field 40° standard color stereo fundus photographs by the Wisconsin Reading Center (University of Wisconsin, Madison, WI). The study included diabetic retinopathy of ETDRS grading levels 35, 43, and 47 at baseline.

Blood–Retinal Barrier Permeability

Using vitreous fluorometry, the leakage of fluorescein into the vitreous was measured with a fluorometer (Ocumetric, Mountain View, CA) at 30 and 60 minutes after fluorescein injection, as described earlier. In brief, the permeability of the blood–retinal barrier was calculated after correction for light loss in the lens, plasma concentration of fluorescein and the diffusion coefficient for fluorescein in the vitreous. In healthy control subjects, the permeability has been found to be ~2 nm/sec (SD 0.5).[10–13] In diabetic patients, the permeability increases correlated with the level of retinopathy and edema, and in diabetic eyes with CSME the permeability in an earlier study with the same method was increased by a factor of 5 (mean, 11.3 nm/sec; 95% CI 9–15).[5] Vitreous liquefaction or posterior vitreous liquefaction[10] interferes with the calculation of blood–retinal barrier permeability, and in a few eyes passive permeability could not be calculated.[14]

Retinal Thickness

Six radial line scans of 6 mm were obtained with optical coherence tomography (model 2000, OCT2; Carl Zeiss Meditec, Inc., Dublin, CA). The software of the instrument could not produce retinal maps calculating mean thickness of specified regions. Thus, the retinal thickness from the six scans were calculated by the algorithm of the original software, and the retinal thickness was averaged off-line for three regions: the foveal region with a 500 μm radius from the center, an inner ring from 500 to 1500 μm from the center and an outer ring from 3000 to 6000 μm from the center. The outer ring was not available at baseline for eight eyes.

Calibers of Retinal Arteries and Veins

Vascular diameter was determined using computerized image analysis software enabling manual definition of the endpoints for analysis of a given retinal vascular segment.[6] Vascular contour tracing was avoided near vascular crossings and branchings, near hard exudate and cotton wool spots, and near strong reflexes from the posterior hyaloid. To allow precise tracking near the ends of the selected segments, the tracking was extended beyond the target segment for a standard length of 40 pixels, so as to provide supporting extrapolation landmarks. This corresponds to about twice the diameter of a large retinal vein at the rim of the optic disc. Vessel calculation was performed in only one eye for each patient and only if image quality was of sufficient quality for the procedure. Right eyes were preferred if photographs of comparable image quality were available for both eyes.

Statistics

For the present analysis, the following visits were included: baseline, 12, 18 and ~3.4 years (all these visits within the ruboxistaurin study), 3.8 years after ruboxistaurin medication and the final follow-up with a mean of 5.7 years. A Cox regression model was used for modeling the hazard for the event of CSME with the inclusion of various covariates: baseline values of HbA_{1c}, mean blood pressure, blood–retinal barrier permeability, retinal thickness, and vessel diameters. The Cox model includes the specific time for examinations and allows interval censoring, meaning that the assumptions for the model do not include that the exact time of the event is known. The model formulation calculates the hazard based on the log of the time variables (complementary log–log model), which is time since baseline and the interval between visits. For all results, the time since baseline, interval since last visit and treatment (placebo or active treatment) during the ruboxistaurin part of the study were included, a correction was included for the nonindependence between eyes.

Remaining covariates were entered in a forward procedure and only maintained if significant at a 5% level. The blood–retinal barrier permeability was log transformed due to a non-normal distribution or entered as a class variable; all other covariates were entered in the original scale. If no other test is mentioned, the appropriate *t*-tests were used to compare variables between groups. Data analysis was performed with commercial software (SAS software package, ver. 8e; SAS Institute, Cary, NC); the genmod procedure was used for the Cox model. The level of statistical significance for all tests was set at 5%.

Results for the effect of ruboxistaurin on the same study population have been presented in an earlier paper,[9] for permeability data a difference of two eyes between the present paper and the previous is due to the event of early photocoagulation which were excluded in the previous paper. However, an exclusion would not be appropriate for the present study.

TABLE 2. Baseline Systemic Characteristics of Patients with Non-CSME DME in Relation to Outcome: No Photocoagulation or Photocoagulation for DME

	Age (y)	Duration (y)	HbA_{1c}* (%)	Mean BP† (mm Hg)
No photocoagulation any eye (n = 27)	51.4 (7.8)	16.7 (8.0)	9.04 (1.1)	99.8 (11.8)
Photocoagulation in one or both eyes (n = 18)	53.2 (9.9)	12.7 (7.3)	9.30 (1.5)	101.4 (9.1)

* HbA_{1c} decreased during the study (P = 0.0001 for comparison of baseline to final follow-up).

† Mean arterial blood pressure did not change significantly during the study ($P > 0.1$ for comparison of baseline to final follow-up).

RESULTS

Of 62 eyes in 45 patients that completed follow-up, 22 eyes in 18 patients had received photocoagulation for CSME between baseline and the last follow-up examination (Tables 1, 2). Of these 22 eyes, 12 eyes in 12 patients underwent photocoagulation treatment during the 3.4-year duration of the interventional study, the treatment being performed within 18 months of baseline in four eyes in four patients. The mean follow-up in the 40 eyes that did not undergo photocoagulation was 5.5 (SD 1.1; range, 3.5–7.3) years. For treated patients, the mean period of observation between baseline and the first photocoagulation session in the first eye was at 3.6 (SD 2.0; range, 0.4–7.3) years.

HbA_{1c} decreased during the study, from 9.3 at baseline to 8.7 at the last visit (P = 0.004) in the 28 cases with a >5-year follow-up, whereas the mean blood pressure increased, but not significantly, from 99.9 to 101.6 mm Hg (P = 0.55).

Comparison of baseline values demonstrated that the mean leakage of 5.6 (95% CI 4.2–7.6) nm/s in eyes that progressed to CSME and photocoagulation was significantly higher than the mean leakage of 3.39 (95% CI 2.7–4.3) nm/s in eyes that did not progress (P = 0.01; Table 3).

The difference was sustained at the 12- and 18-month follow-up (Table 3). Differences at baseline between the two groups in retinal thickness, central retinal artery equivalent diameter, central retinal vein equivalent diameter, HbA_{1c}, and mean arterial blood pressure were numerically small and did not reach statistical significance ($P > 0.1$; Table 3).

The increase in retinal thickness that led to the diagnosis of CSME and photocoagulation treatment was detectable 18 months after baseline (analysis excluding the four eyes that had undergone photocoagulation at this time; Table 3). The difference was modest in the foveal field (P = 0.06) and significant for the inner perifoveal ring (P = 0.04).

The statistical effects of age, gender, duration of diabetes, HbA_{1c}, and blood pressure were evaluated with a Cox proportional hazards model. The basic model incorporated time from baseline, interval between visits, and a correction factor for the effect of ruboxistaurin treatment. Although the effect of treatment was nonsignificant, it was considered an essential explanatory variable of the statistical model because it was given for approximately half of the study period and because larger studies have shown benefit of treatment in DME. The conclusion was unaltered, whether treatment was included or excluded from the analysis. Baseline blood–retinal barrier permeability was found to be significant, both as a continuous variable and as a class variable. The hazard ratio for eyes with permeability higher than 3.6 (median of baseline value) nm/s was 6.2 times (95% CI 1.7–23.2; P = 0.0043) higher than that of eyes with baseline permeability lower than or equal to 3.6 nm/s. Setting the threshold for binary division to 2.98 nm/s, which is the upper range limit of leakage seen in healthy subjects (mean plus 2 SD), the hazard ratio was 13.7 (95% CI 1.7–111; P = 0.0022).[10] No contributory value in outcome modeling was found for blood pressure, HbA_{1c}, or retinal thickness. Not surprisingly, all measures of retinal thickness at 18 months, when significant thickening of the group of progressing eyes relative to the group of nonprogressing eyes had occurred, were found to be predictive of the outcome photocoagulation for CSME (P = 0.02 or lower).

Treatment with ruboxistaurin had no statistically significant effect on the rate of progression to photocoagulation treatment during or after the controlled intervention. Baseline ETDRS retinopathy levels ranged from 35 to 47 (27 eyes at level 35, 24 eyes at level 43, and 10 eyes at level 47). Baseline permeability was significantly correlated with baseline retinopathy level (P = 0.001, analysis of variance). Baseline retinopathy was not independently significant for the outcome of photocoagulation ($P > 0.2$). The lack of significance was probably due to the less sensitive semiquantitative scale of retinopathy compared with the permeability, which is measured on a quantitative scale and more closely related to the pathophysiology of macular edema.

DISCUSSION

Diabetic macular edema develops slowly and although systemic risk factors for development and progression of DME are known (increasing duration of diabetes, increasing HbA_{1c} and, possibly, increasing arterial blood pressure), these factors, alone or combined, permit no certain prediction of which eyes will progress from retinopathy without edema to DME or from lesser grades of DME to CSME.

In the present study, we followed eyes with nonclinically significant DME for up to 7 years. In eyes that eventually progressed to CSME, blood–retinal barrier permeability increased significantly from baseline through month 18. In contrast, retinal thickness was comparable between nonprogressing and progressing eyes, from baseline and up to month 18. Retinal artery and vein diameters were comparable in nonprogression and progressing eyes from baseline through month 18 and up to the final study visit.

The use of fluorescein angiography and in particular the quantitative assessment of leakage with vitreous fluorometry have diminished in recent years due to the introduction of noninvasive measurement of retinal thickness and retinal vessel diameters. However, the results of the present study indicate that the initial stages of edema formation is related to basic mechanisms of the blood–retinal barrier while morphologic changes are later events and the reluctance to use invasive fluorescein angiography and related quantitative methods may lead to an incomplete understanding of the underlying mechanisms of edema progression.

Age, duration of diabetes, HbA_{1c} and arterial blood pressure failed to reach statistical significance as predictors of progression to photocoagulation (Cox's regression model), leaving blood–retinal barrier leakage as the only significant factor, the hazard ratio being 6.3 times higher for leakage values above 3.6 nm/s than for values lower than or equal to 3.6 nm/s (median of baseline values). The present study is not ideal, as it included

TABLE 3. Ocular Characteristics in Eyes with Non-CSME DME at Baseline in Relation to Outcome: No Photocoagulation or Photocoagulation for DME

	Baseline	18 Months	Final Study Visit
Final	−PC/+PC	−PC/+PC	−PC/+PC
Best corrected visual acuity (letters)			
Eyes (*n*)	40/22	40/18	38/10
Acuity (mean)	86/87	87/89	81/81
95% CI	85–88/84–89	72–99/76–91	86–86/75–87
P	NS	NS	NS
Eyes censored after photocoagulation	—	4 eyes	11 eyes
Eyes lost to follow-up			3 eyes
Permeability (nm/s)			
Eyes (*n*)	38/19	38/17	29/8
Mean	3.39/5.62	3.02/5.37	3.55/5.50
95% CI	1.7–4.3/4.2–7.6	2.4–3.8/3.9–7.4	2.6–4.8/3.4–8.9
P	0.01	0.005	0.3
Retinal thickness (um)			
Eyes (*n*)	39/21	34/16	33/9
Foveal field (mean)	199/206	207/224	198/233
95% CI	192–207/190–223	198–216/206–243	187–208/205–261
P	NS	0.06	0.1
Inner (perifoveal) ring			
Mean	264/266	267/279	265/274
95% CI	258–270/257–275	262–274/271–288	256–274/260–289
P	NS	0.04	NS
Outer ring			
Mean	237/240	248/257	243/254
95% CI	228–245/232–247	242–255/241–269	235–251/234–269
P	NS	NS	0.09
Central retinal artery equivalent			
Eyes (*n*)	25/11	23/9	21/6
Diameter (mean)	162/155	168/156	160/154
95% CI	157–168/137–178	162–174/140–172	155–165/147–161
P	NS	NS	0.07
Central retinal vein equivalent			
Eyes (*n*)	25/11	23/9	21/6
Diameter (mean)	275/284	288/282	276/265
95% CI	268–283/261–286	278–298/269–294	267–283/233–296
P	NS	NS	NS

The study population of patients with DME of grade less than CSME was classified by outcome: no progression to CSME and photocoagulation (−PC) or progression to CSME and photocoagulation (+PC) within the mean follow-up of 5.65 years. Best corrected visual acuity expressed as ETDRS letters (85 letters = 20/20 Snellen). Permeability of the blood-retinal barrier was assessed by vitreous fluorometry, retinal thickness by optical coherence tomography, and retinal trunk vessel diameter (central artery and vein diameter equivalent) by computerized analysis of digital fundus photographs. Eyes were censored from the table after photocoagulation had been performed. Statistical significance was calculated with two-sided *t*-tests comparing no progression to CSME with progression to CSME and photocoagulation. Only differences amounting to $P \leq 0.1$ are specified. For simplicity, the 12-month data have been omitted. The probabilities at 12 months did not differ significantly from baseline.

an intervention study in the initial stage. To correct for this, the analysis included correction for the potential effects of the experimental intervention during the first 3.4 years of the study (ruboxistaurin 4, 16, or 32 mg/d versus placebo, 25% allocated to placebo) during the first 3.4 years of the study. Half of the progressing eyes progressed within the duration of the study.

The lack of significance of ruboxistaurin is not in contrast to the positive effect of treatment found in relevant subgroups of the main study[8] as the number of patients in the present substudy was small and the power thus insufficient. However, permeability seems to be a sensitive parameter, as a previous paper based on the same population as in the present substudy showed that ruboxistaurin treatment interacted significantly with baseline permeability (i.e., permeability decreased during the treatment time in eyes with high baseline permeability).[9] Thus, the time to progression to photocoagulation may have been prolonged in treated eyes with high baseline permeability and, as mentioned, a correction for treatment was incorporated in the calculation of the hazard.

The observation that between-baseline leakage rather than baseline retinal thickness predicted a strongly thickness-related outcome—namely, photocoagulation for CSME—is surprising. It suggests that no simple relation exists between blood–retinal barrier leakage and retinal edema. In agreement with this, we have found indications that the rate of elimination of fluorescein from the retina is upregulated in concert with the increased retinal vascular leakage in diabetic retinopathy.[4]

Additional factors that need to be considered are the barrier effects of various retinal layers, and the effects of abnormalities in intraretinal hydrostatic and colloid osmotic pressure in DME. In addition, the role of aquaporins and the expansibility of the retinal layers may be relevant in the formation and localization of edema.

In conclusion, we have identified blood–retinal barrier leakage as a significant risk factor for the progression of lesser grades of DME to photocoagulation for CSME. Retinal thicken-

ing was markedly delayed in relation to the increase in leakage and first found to statistically significant 18 months after the baseline examination had revealed increased blood–retinal barrier permeability.

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