

Opportunities for Therapeutic Intervention: Molecular Biology of Diabetic Retinopathy

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DIABETIC MICROVASCULAR COMPLICATIONS TODAY.

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STATEMENT OF NEED

The risk of death among people with diabetes is about two times that of those without the disease. The annual cost in the United States alone is more than \$132 billion dollars.

Diabetic retinopathy develops in virtually 100% of patients with type 1 diabetes and 60% of those with type 2. Within 10 years of diabetes onset, 20% of type 1 diabetic patients and 14% of type 2 diabetics develop diabetic macular edema. It is the leading cause of moderate vision loss in diabetes.

TARGET AUDIENCE

This activity is designed for ophthalmologists, specifically retinal specialists and others who treat diabetic retinopathy.

LEARNING OBJECTIVES

After successful completion of this program the participants should be able to:

- describe the molecular biology underlying diabetic retinopathy;
- identify therapeutic strategies for intervention; and
- discuss the DRCR.net initiatives.

METHOD OF INSTRUCTION

Participants should read the learning objectives and CME program in their entirety. After reviewing the material, they must complete the self-assessment test, which consists of a

series of multiple-choice questions.

Participants have a choice of completing this activity online by visiting www.DiabeticMCToday.com; getting real-time results at www.CMEToday.net; or by using the print forms following this activity.

ACCREDITATION

This activity has been planned and implemented in accordance with the essentials and standards of the ACCME through the joint sponsorship of The Dulaney Foundation and *DIABETIC MICROVASCULAR COMPLICATIONS TODAY.*

DISCLOSURE

In accordance with the disclosure policies of The Dulaney Foundation and to conform with ACCME and FDA guidelines, all program faculty are required to disclose to the activity participants: 1) the existence of any financial interest or other relationships with the manufacturers of any commercial products/devices, or providers of commercial services that relate to the content of their presentation/material or the commercial contributors of this activity; and 2) identification of a commercial product/device that is unlabeled for use or an investigational use of a product/device not yet approved.

FACULTY DISCLOSURE DECLARATIONS

Lloyd Paul Aiello, MD, PhD, serves as a consultant to and

receives honoraria from Eli Lilly and Company and Eyetech.

FACULTY CREDENTIALS

Dr. Aiello is associate professor of ophthalmology at Harvard Medical School, director of the Beetham Eye Institute and head of the section on eye research at Joslin Diabetes Center, Boston.

INTRODUCTION

Diabetic retinopathy exists across a spectrum of stages and severity, and macular edema can occur at any point across the spectrum. Even before diabetic retinopathy is evident, biochemical changes begin.¹

Hyperglycemia plays a role with regard to cellular dysfunction and damage, with a host of complex processes that come into play. Hyperglycemia is the principal underlying cause of the complications of diabetes.

PKC

Protein kinase C (PKC) activation appears to be a key player in many of these pathways.² PKC inhibition may help ameliorate hyperglycemia-induced cellular dysfunction. The PKC enzyme modulates molecules in the body and the beta isoform is primarily involved in the complications of diabetes and other vascular issues.³ In the state of hyperglycemia diacylglycerol is synthesized, which in turn activates PKC. Therefore, the fundamental problem is that PKC is activated in the diabetic condition.

The level of PKC activity then correlates to the severity of diabetic retinopathy. PKC-beta activation plays a key role in mediating early retinal changes in diabetes. Even when PKC beta was overexpressed in nondiabetic mice, retinal abnormalities were seen.⁴

VEGF

Growth factor mediation – particularly vascular endothelial growth factor (VEGF) – is part of retinopathy. Proliferative diabetic retinopathy appears to be driven by ischemia in the retina. Growth factors in the eye lead to neovascularization.

VEGF is a 46 kDa homodimeric glycoprotein that was first identified in vascularized tumors. VEGF is produced by many ocular cell types and is induced by hypoxia. It binds to high-affinity receptors on retinal endothelial cells and stimulates retinal endothelial cell growth. VEGF promotes vascular permeability and induces disassociation of tight junction components.

Retinal VEGF levels are elevated in diabetes and VEGF can cause diabetic retinopathy-like changes in the eye.⁵⁻⁷ Data shows that VEGF plays a key role in mediating retinal neovascularization and vascular permeability under

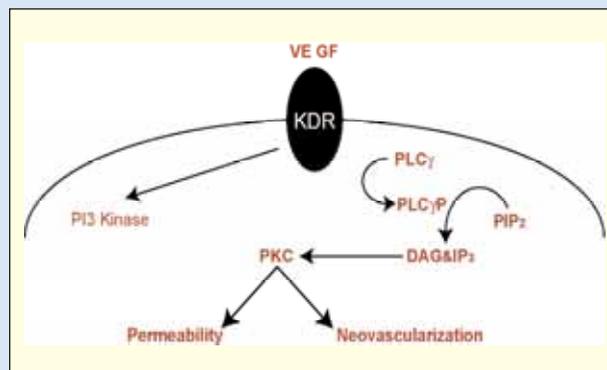


Figure 1. Mechanism of VEGF action.

ischemic retinal conditions, such as diabetic retinopathy.

If an agent was able to block VEGF, we might be able to prevent diabetic complications in the eye. VEGF inhibition in mice and primate models was shown to be successful.^{8,9}

ANTI-VEGF THERAPIES

Current anti-VEGF approaches that are being studied include pegaptanib sodium, ranibizumab, VEGF trap and bevacizumab.

- Pegaptanib sodium (Macugen, Eyetech) acts as an intravitreal aptamer VEGF inhibitor. Its potential indication is diabetic macular edema (DME) treatment and it is in phase 3 trials. It has been Food and Drug Administration (FDA) approved for the treatment of neovascular or wet age-related macular degeneration.

- Ranibizumab (Lucentis, Genentech) acts as an intravitreal humanized anti-VEGF antibody fragment. Its potential indication is DME and studies are planned. Genentech will soon seek FDA approval for the indication of the treatment of neovascular or wet age-related macular degeneration.

- The VEGF Trap (Regeneron) is an intravitreal VEGF receptor construct that is being studied for the potential treatment of DME.

- Bevacizumab (Avastin, Genentech) is an intravitreal anti-VEGF antibody that is being studied in DME treatment, off-label. Currently it is approved as first-line treatment for patients with colorectal cancer that has spread to other parts of the body. Mechanism of VEGF action is shown in Figure 1.

PKC-BETA INHIBITION

Another therapeutic target option would be to block PKC-beta activation along the pathway. A PKC-beta inhibitor should be highly PKC selective, highly beta isoform selective and orally bioavailable. Eli Lilly and Company's ruboxistaurin, currently in development, is all of these things. In clinical studies it has been shown to reduce

TABLE 1. ANTI-SIGNALING APPROACHES

Agent	Action	Indication	Status
PKC-beta inhibitor OTDRS (ruboxistaurin)	Oral, PKC-beta inhibitor	RBF MCT	Phase 1b completed
PKC-beta inhibitor DRS1 (ruboxistaurin)	Oral, once-daily PKC-beta inhibitor	PDR NPDR	Phase 2/3 completed
PKC-beta inhibitor DMES (ruboxistaurin)	Oral, once-daily PKC-beta inhibitor	DME	Phase 2/3 completed
PKC-beta inhibitor DRS2 (ruboxistaurin)	Oral, once-daily, PKC-beta inhibitor	Visual loss	Phase 3 completed
PKC-beta inhibitor DMES2 (ruboxistaurin)	Oral, once-daily, PKC-beta inhibitor	DME	Phase 3 enrolling

retinal neovascularization, reduce diabetes-induced permeability and normalize retinal blood flow.

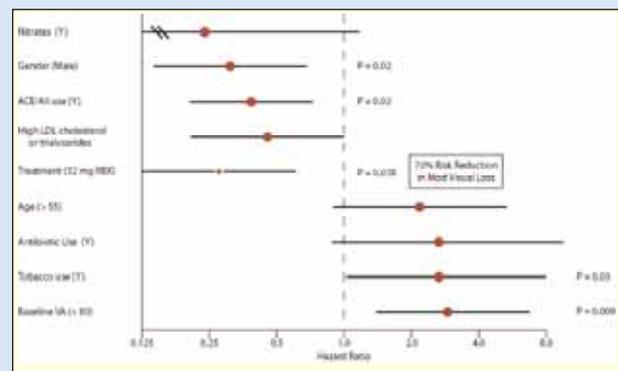
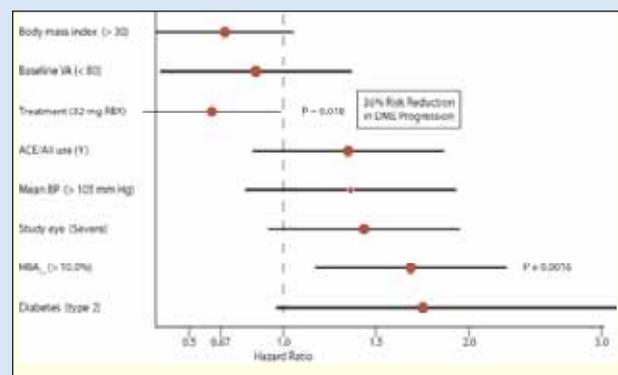
A ruboxistaurin phase 2/3 trial studied 252 total patients in the diabetic retinopathy arm and 426 total patients in the DME arm. In the diabetic retinopathy arm, the overall goal of the trial was to see if the agent slowed the progression of nonproliferative diabetic retinopathy or prevented laser treatment. Diabetic retinopathy severity was 47b to <53e (moderate to severe nonproliferative diabetic retinopathy [NPDR]). Patients had no prior panretinal photocoagulation but could have prior focal treatment and could have macular edema at baseline (Table 1).

In the DME study the outcome was the slowing or reversal of the progression of macular edema or to prevent laser treatment. The diabetic retinopathy severity was 20 to ≤47 a (mild to moderate NPDR). Patients did not have prior panretinal photocoagulation or focal treatment and did have macular edema at baseline. The Cox proportional hazard model is as shown in Figure 2.

The primary study endpoint in the PKC-DRS was the progression of diabetic retinopathy, meaning a 3+ step change in the ETDRS retinopathy person severity scale (patients with two eyes <level 61) or 2+ step change in the ETDRS retinopathy eye severity scale (patients with only one eye <level 61) or panretinal photocoagulation. The Cox proportional hazard model for moderate visual loss is shown in Figure 3.

The PKC-beta inhibition study showed that ruboxistaurin decreased the development of macular edema threatening the center of the macula and the occurrence of visual loss. Ruboxistaurin did not prevent the progression of dia-

betic retinopathy nor the combined outcome of DME progression or the application of laser photocoagulation. Two large, high-power, single-dose trials evaluating the impact of ruboxistaurin on these endpoints are underway (Figure 4).



Figures 2 and 3. Cox proportional hazard models for DME progression PKC DMES (top) and moderate visual loss.

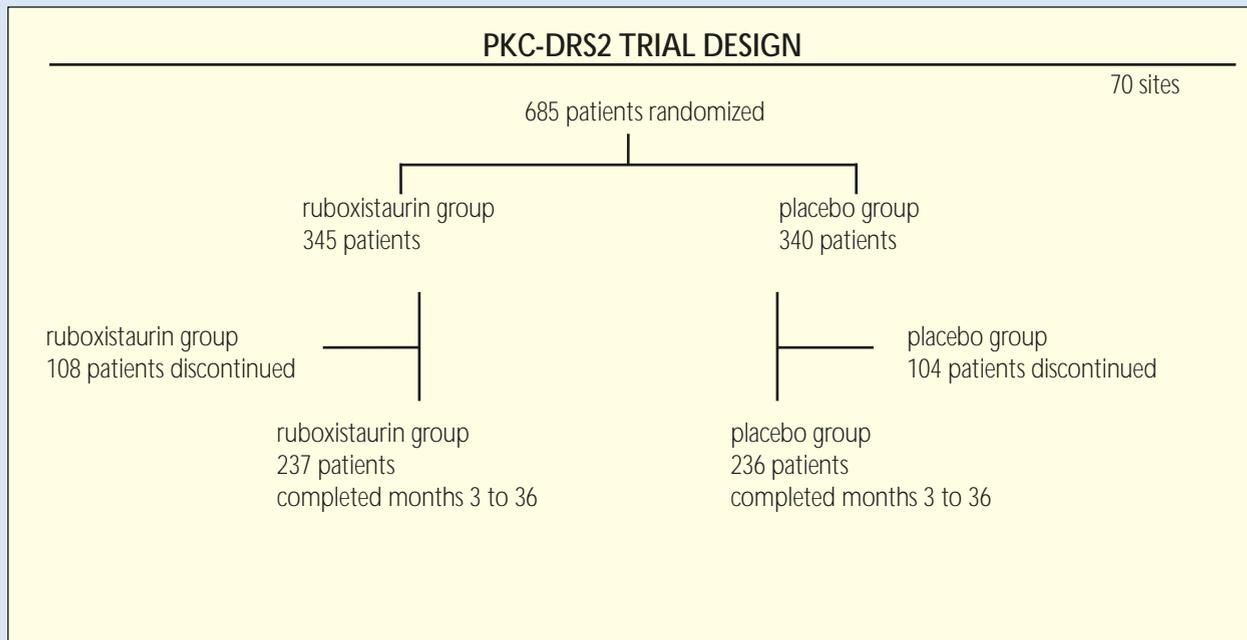


Figure 4. PKC-DRS2 phase 3 trial design.

INTRAVITREAL STEROIDS

The potential of intravitreal steroid treatment is that it can reduce retinal edema, but there is a time limited effect. The visual acuity response of this method of treatment does not always commensurate with reduction in retinal edema. Intravitreal steroids come with a high risk of cataract, intraocular pressure elevation and endophthalmitis, and often it is an off-label use.

Intravitreal steroids are being investigated as part of the Diabetic Retinopathy Clinical Research Network. The DRCR.net is dedicated to multicenter clinical research of diabetic retinopathy, macular edema and associated disorders. (To read more about DRCR.net, see page 29 of the November/December 2005 issue of *DIABETIC MICROVASCULAR COMPLICATIONS TODAY*.)

Triamcinolone acetonide is being studied in a phase 3, multicenter, randomized clinical trial. The treatment is delivered as a preservative-free, pH balanced solution, in a single-dose prefilled syringe. Patients are randomized to one of three treatment groups: standard of care (conventional treatment including modified ETDRS photocoagulation), intravitreal injection of 1 mg triamcinolone acetonide or intravitreal injection of 4 mg triamcinolone acetonide.

The injection volume is always 0.05 mL and follow-up is 3 years. The first patient was enrolled in July 2004 and more than 417 patients have been enrolled to date. The goal is 690 patients total. I recently presented this information at the 2005 American Academy of Ophthalmology Annual meeting in Chicago.¹⁰

CONCLUSION

Diabetes is a systemic disease. Diabetic retinopathy is the leading cause of blindness in working age adults. Diabetic neuropathy is the leading cause of nontraumatic amputations and diabetic nephropathy is the leading cause of end-stage renal disease. Also, with regard to the macrovascular picture, patients with diabetes have a two- to fourfold increase in cardiovascular mortality and stroke. ■

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4. Wakasaki H, Koya D, Schoen FJ, et al. Targeted overexpression of protein kinase C beta2 isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci U S A*. 1997;94:9320-9325.
5. Aiello, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med*. 1994;331:1480-1487.
6. Adams AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol*. 1994;118:445-450.
7. Quam T, Xu O, Jousseaume AM, et al. VEGF-initiated blood retinal barrier breakdown in early diabetes. *Invest Ophthalmol Vis Sci*. 2001;42:2408-2413.
8. Tolentino MJ, Miller JW, Gragoudas, et al. Intravitreal injections of VEGF produce retinal ischemia and microangiopathy in adult primates. *Ophthalmology*. 1996;103:1820-1828.
9. Tolentino MJ, McLeod DS, Taomoto M, et al. Pathologic features of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. *Am J Ophthalmol*. 2002;133:373-385.
10. Aiello LP. Molecular biology of diabetic retinopathy: Opportunities for therapeutic intervention. CME Symposium presented during the the 2005 American Academy of Ophthalmology meeting, October 15, 2005. Chicago.

CME QUESTIONS

Circle the most appropriate answer in the "ANSWER SECTION" on the following page.

1. What is the principal underlying cause of the complications of diabetes?
 - a. hyperglycemia
 - b. growth factors
 - c. ischemia
 - d. PKC-beta

2. What PKC isoform is primarily involved in the complications of diabetes and other vascular issues?
 - a. alpha
 - b. beta
 - c. gamma
 - d. delta

3. Which of the following statements is NOT true about VEGF?
 - a. It is a glycoprotein
 - b. It was first identified in vascularized tumors
 - c. It binds to high-affinity receptors on retinal endothelial cells and stimulates retinal endothelial cell growth
 - d. Retinal VEGF levels are decreased in diabetes

4. Which of the following is NOT a VEGF therapy that was discussed in the activity?
 - a. Macugen
 - b. Lucentis
 - c. triamcinolone acetonide
 - d. Avastin
 - e. VEGF Trap

5. Which of the following is NOT true with regard to ruboxistaurin?
 - a. it is highly PKC selective
 - b. it is highly beta isoform selective and orally bioavailable
 - c. it has been shown to reduce retinal neovascularization
 - d. it has not been studied in patients with existing macular edema

6. Which of the following is NOT a complication associated with intravitreal steroid use discussed in the activity?
 - a. limited time of effect
 - b. increased risk of cataract
 - c. increased in IOP
 - d. loss of tear production

7. Intravitreal steroids are being investigated in the DRCR.net.
 - a. true
 - b. false

8. The goal number of patients in the steroid trial mentioned is:
 - a. 2004
 - b. 417
 - c. 690
 - d. 222

9. Diabetes is not a systemic disease.
 - a. true
 - b. false

10. What is the leading cause of blindness in working age adults?
 - a. diabetic retinopathy
 - b. diabetic neuropathy
 - c. diabetic nephropathy
 - d. CVD and stroke

REGISTRATION/EVALUATION FORM: MOLECULAR BIOLOGY OF DIABETIC RETINOPATHY

To obtain AMA/PRA category 1 credit, you must:

- Read the learning objectives and the CME article and complete the self-assessment test.
- Photocopy and complete this registration/evaluation form and record your test answers in the Answer Section below.
- Send the Registration/Evaluation form to **The Dulaney Foundation, PO Box 44408, Phoenix, AZ 85064, or fax to 602-508-4893.**
- Retain a copy of your test answers. Your answer sheet will be graded, and if you achieve a passing score of 70% or better, you will receive a CME credit letter awarding AMA/PRA category 1 credit within 4 weeks. If you do not achieve a passing score, you will be notified and offered the opportunity to complete the activity again.

ANSWER SECTION

Circle the best answer for each question on page 41.

1. A B C D 2. A B C D 3. A B C D 4. A B C D E 5. A B C D
6. A B C D 7. A B 8. A B C D E 9. A B 10. A B C D

REGISTRATION FORM

First name _____ Last name _____ Degree (MD, PhD) _____

Specialty _____

Institution or practice name _____

Address _____

City _____ State _____ Zip Code _____ Country _____

Telephone _____ Fax _____ E-mail address _____

The processing fee has been underwritten by an educational grant from Eli Lilly and Company.

I attest that I have completed this activity as designed and I am claiming ____ (up to 1 credit) AMA/PRA category 1 credit.

Signature _____ Date _____

Credit for this activity is available until Jan. 31, 2007.

The planning and execution of useful and educationally sound continuing education activities are guided in large part by input from participants. Please assist us in evaluating the effectiveness of this activity and make recommendations for future educational offerings by completing this evaluation form. Your response will help ensure that future programs are informative and meet the educational needs of all participants. Please note: CME credit letters and long-term credit retention information will only be issued upon receipt of this completed evaluation. Thank you for your cooperation.

OBJECTIVES

After successful completion of this program, you should be able to:

- | | | | | | |
|--|---|---|---|---|---|
| • describe the molecular biology underlying diabetic retinopathy | 5 | 4 | 3 | 2 | 1 |
| • identify therapeutic strategies for intervention | 5 | 4 | 3 | 2 | 1 |
| • discuss the DRCR.net initiatives | 5 | 4 | 3 | 2 | 1 |

(Please circle the number that is most accurate; 5 represents strongly agree and 1 represents strongly disagree.)

OVERALL EVALUATION

- | | | | | | |
|--|---|---|---|---|---|
| • The information presented increased my awareness/understanding of the subject. | 5 | 4 | 3 | 2 | 1 |
| • The information presented will influence how I practice. | 5 | 4 | 3 | 2 | 1 |
| • The information presented will help me improve patient care. | 5 | 4 | 3 | 2 | 1 |
| • The faculty demonstrated current knowledge of the subject. | 5 | 4 | 3 | 2 | 1 |
| • The program was educationally sound and scientifically balanced. | 5 | 4 | 3 | 2 | 1 |
| • The program avoided commercial bias or influence. | 5 | 4 | 3 | 2 | 1 |
| • Overall, the program met my expectations. | 5 | 4 | 3 | 2 | 1 |
| • I would recommend this program to my colleagues. | 5 | 4 | 3 | 2 | 1 |

(Please circle the number that is most accurate; 5 represents strongly agree and 1 represents strongly disagree.)

• If you anticipate changing one or more aspects of your practice as a result of your participation in this activity, please provide a brief description of how you plan to do so: _____

• Please provide any additional comments pertaining to this activity (positive and negative) and suggestions for improvements: _____