The Critical role of Retinal Pigment Epithelium in the Pathogenesis of Diabetic Retinopathy

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The Pathophysiology of Diabetic Retinopathy

- Most of the research on the pathophysiology of diabetic retinopathy has been focused on the impairment of the neuroretina and the breakdown of the inner BRB since this is where clinical lesions are manifested.
Pathological changes during diabetic retinopathy. Formation of pericyte ghosts (A) and acellular capillaries (C) and in diabetic retinopathy. Normal architecture of blood vessel containing endothelial cell surrounded by pericyte (B).
Hypermelcemia is the primary pathogenic factor in the development of diabetes complications

- Chronic exposure of the retina to the hyperglycemia is known to be the primary pathogenic factor in the development of diabetes complications

protein kinase C (PKC), poly(ADPribose)polymerase (PARP), advanced glycation end products (AGEs)
Development of Extensive Microvascular Pathologies in the Retinal but not Cerebral Capillaries

- Although brain and retina are embryologically similar, and both vascular beds are exposed to similar concentrations of blood glucose, the extensive microvascular pathology develops only in the retinal capillaries, not in the cerebral capillaries!!!
Table 1  Differences between retinal and brain capillaries of 10 diabetic and normal dogs. Duration of diabetes: 5 years

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Retina</th>
<th>Brain</th>
<th>Normal retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microaneurysms</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pericyte ghosts</td>
<td>25</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Acellular capillaries</td>
<td>195</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thickness of basement membrane (nm)</td>
<td>237</td>
<td>171</td>
<td>135</td>
</tr>
</tbody>
</table>

From Kern and Engermann. The original contains the statistics and similar data on other dogs made diabetic with galactose.
Glucose Transport in Neuroretina

• The neuroretina, which is among the most metabolically active tissues in the body, is nourished by transport of glucose across the endothelial cells of the capillaries of the inner BRB and from the choroidal vessels across the retinal pigment epithelium of the outer BRB
Comparison of glucose influx in retina and brain of diabetic rats

• Glucose influx in retina exceeded that of cortex by about threefold for the nondiabetic

• The glucose levels in the diabetic retina were fourfold to sixfold greater than the nondiabetic retina.

• The cortical glucose levels remained unchanged.

Glucose Transporters

The initial step in the delivery of glucose to the retina and brain is its transport across the blood–retinal barrier (BRB) or the blood–brain barrier (BBB) by the major glucose transporter, GLUT-1.

The facilitated transport of glucose across the plasma membrane is effected by a family of proteins known as the Glut proteins, of which at least eight members are known.
• Glucose may gain entry into the endothelial cells of the inner BRB only via transport mediated by GLUT1.

• GLUT1-mediated transport operates at near-saturation levels at normal physiological blood glucose concentrations.

• Therefore, elevations in blood glucose levels, such as those seen in diabetes mellitus, will have only a minor impact in increasing intracellular glucose concentrations.
• GLUT-1 was significantly decreased in neural retina of rats with streptozotocin-induced diabetes, whereas brain and RPE were unaffected.

• Despite less expression of glucose transporter in diabetics, studies have found that retinal glucose tissue concentrations increased by greater than threefold compared with nondiabetics.

The RPE and Outer Blood Retina Barrier

• RPE contains high amounts of glucose transporters in both the apical and the basolateral membranes.

• It is estimated that a significant amount of all glucose (about 60%) enters the retina via the RPE.

• Both GLUT1 and GLUT3 are highly expressed in the RPE.

• GLUT3 mediates the basic glucose transport.

• GLUT1 is responsible for inducible glucose transport in response to different metabolic demands.

We hypothesized that the increase in retinal glucose levels during diabetes is from increased entry into retina at the RPE–choroid interface through glucose-6-phosphatase (G6P)-mediated activity of RPE cells.
Detection of G6Pase in RPE cell culture

G6Pase

Actin
G6 Pase expression in RPE
Within a cell, glucose 6-phosphate is produced by phosphorylation of glucose on the sixth carbon.

Glucose 6-phosphatase (G6Pase) is an enzyme that hydrolyzes glucose-6-phosphate resulting in the creation of a phosphate group and free glucose.

Glucose is then exported from the cell via glucose transporter membrane proteins. In humans, there are three isozymes, G6PC, G6PC2, and G6PC3.
• G6Pase is found mainly in the liver and the kidney, plays the important role of providing glucose during starvation and plays a key role in the homeostatic regulation of glucose levels.
• Mutations in the G-6-Pase gene that result in the complete absence of enzymatic activity have been identified in humans as the molecular cause for glycogen storage disease (GSD) type Ia von Gierke's disease.

• GSD is characterized by hypoglycemia, increased serum lactate levels, and excessive glycogen storage in the liver.
Proposed role of RPE in the Development of Diabetic Retinopathy

Diabetes cause a 2-3 fold increase in G6Pase activity. Insulin causes a decrease in the activity of G6Pase.
Pericyte

Endothelial cell

Photoreceptor

A

HGT  PC  EC  661W

Ubiquitin
Thielavins as glucose-6-phosphatase (G6Pase) inhibitors
• TSP1−/− male mice were made diabetic with a single intraperitoneal (i.p.) injection of streptozotocin (STZ; Sigma, St. Louis, MO; 180 mg/kg).

• STZ-injected animals were considered diabetic when blood glucose levels reached stable levels over 250 mg/dl.
• Mice after 1 month of diabetes were intravitreally injected with 2 µL glucose-6-phosphatase (G6Pase) inhibitors or DMSO and repeat after 6 weeks

• Diabetic mice were sacrificed after 3 months of diabetes and the eyes were collected. Blood glucose levels were measured with a portable glucometer using a drop of blood collected from a small incision at the tip of the tail.
Nuclear morphology was used to distinguish pericytes from endothelial cells.

The nuclei of endothelial cells are oval or elongated and lie within the vessel wall along the axis of the capillary, while pericytes nuclei are small, spherical and stain densely and generally have a protuberant position on the capillary wall.
EC/PC ratio

Control

Treated

p<0.01

Endothelial / Pericyte cells ratio
Conclusion

• In Diabetes, significant amount of glucose enters the retina via the RPE, and these estimates are consistent with the presence of appreciable amounts of Glucose-6-Phosphatase in the RPE.
Future direction

**Long term**
Synthesis of new competitive antagonist
Develop Slow release polymer-drug systems

**Short term**
Optimizing subthreshold laser for PRP
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