

Joslin Diabetes Center White Paper
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PKC Research at Joslin Diabetes Center – 1981 – 2005

A summary of more than 20 years of study dedicated to understanding the molecular mechanisms by which hyperglycemia damages vascular tissues, and testing PKC inhibitors to prevent the long-term complications of diabetes.

One of the most difficult tasks for diabetes patients is controlling the level of glucose in their blood. As clinical studies have shown, abnormally high glucose, or hyperglycemia, over time causes a host of serious health problems, including diseases of the kidney, retina, nerves and heart. All long-term diabetic complications – which affect people with both type 1 and type 2 diabetes – begin with damage to blood vessels.

Since the early 1980s, George L. King, M.D., Director of Research and Head of the Section on Vascular Cell Biology at Joslin Diabetes Center, has studied the role of PKC in the development of vascular (blood vessel) dysfunction. One of his lab's most significant efforts over the past two decades has been to understand the molecular mechanisms by which hyperglycemia damages small blood vessels and to find drug targets to prevent or delay the progression of diabetic vascular diseases.

Joslin's major contributions to PKC research include:

1. Making the original observation that PKC is activated by hyperglycemia
2. Identifying the first isoform, PKC-beta
3. Publishing the first studies showing that a PKC inhibitor can prevent diabetes-induced vascular dysfunction
4. Providing the first evidence that vascular endothelial growth factor (VEGF) increases PKC *in vivo*, leading to blood vessel abnormalities, which improved after treatment with the PKC inhibitor
5. Demonstrating for the first time that PKC-beta is persistently activated in the large blood vessel tissues of the heart and aorta
6. Publishing the first evidence that PKC is involved in adhesion of white cells to blood vessel walls, and that treatment with the PKC inhibitor prevented adhesion and decreased oxidative stress

This paper examines these and other milestones of PKC research at Joslin over the past 20 years, and briefly explains how diabetes affects the kidney and retina.

Understanding Diabetic Kidney Disease

When functioning normally, kidneys remove urea and other wastes from the blood. As hyperglycemia damages the microvessels of the kidney, however, this normal filtering process goes awry. Tiny capillaries known as glomeruli become so porous they allow large proteins such as albumin to pass into the urine (an early sign of kidney damage). Blood vessel walls thicken, increasing blood pressure in the kidney and the whole body. The kidney may become so impaired that it can no longer filter wastes from the body.

Each year, nearly 100,000 people in the United States are diagnosed with kidney failure, and 50 percent of those with end-stage kidney disease have diabetes. Currently, dialysis and kidney transplantation are the only treatments, but both are expensive and reduce a patient's quality of life. That's one reason researchers at Joslin have set their sights on finding a drug target – such as PKC – to delay the onset of symptoms or prevent diabetic kidney disease and other complications altogether.

What Is PKC?

Protein kinase C (PKC) is an enzyme that triggers a chemical reaction by adding a phosphate molecule to another enzyme, a process called phosphorylation, which is essential to the normal production of energy in the body. PKC belongs to a family of enzymes that can regulate many blood vessel functions. It comprises about a dozen different molecular forms, or isoforms, including PKC-alpha, PKC-beta 1 and beta 2 and PKC-delta.

Milestones in PKC Research at Joslin

Thanks to the groundbreaking work of scientists like Judah Folkman, M.D., of Children's Hospital Boston and Michael Gimbrone, M.D., of Brigham and Women's Hospital in Boston, new techniques for culturing blood vessel cells began to emerge in the mid-1970s to 1980s. Building on these techniques, the King lab worked for two years to create conditions that would mimic diabetes *in vivo*, by establishing blood vessel cells in culture and then exposing them to glucose or insulin to observe how the cells were adversely affected – effectively creating the first blood vessel model for diabetes in cultured cells. To understand the molecular events that cause diabetic vascular diseases,

the researchers then asked, what cellular communication pathways are affected by high glucose levels?

After multiple studies, Dr. King and his team hypothesized that activation of PKC – especially the beta isoform – is one of the major signaling pathways stimulated by hyperglycemia. In the late 1980s, using cultured cells from the inner walls of blood vessels of the retina, Dr. King and his colleagues demonstrated that elevated levels of glucose increased PKC activity through a substance called DAG (diacylglycerol), an activator of PKC. In diabetic animal models, the lab also showed that abnormal activation of PKC is an important factor in decreasing blood flow to the retina. These seminal discoveries established the link between hyperglycemia, PKC and diabetic vascular disease.

Following these studies, researchers at Joslin sought to discover whether PKC activation involves only the eye, or also affects the kidney, heart or large arteries. In one series of experiments, the lab determined that several PKC isoforms were activated in different tissues, and showed for the first time that one isoform in particular – PKC-beta – was persistently activated in the large and small blood vessels and heart. In further research, published in 1994, the King lab used cultured cells and animal models to show that hyperglycemia chronically activates the DAG-PKC pathway in blood vessels, further demonstrating the role of PKC in the development of diabetic vascular complications, which takes many years to develop.

Designing a PKC Inhibitor

Finding the key PKC isoform implicated in blood vessel damage was critical to designing a selective inhibitor, a drug that would block PKC-beta but not interfere with normal cell functioning. Because PKC is a vital enzyme, if all isoforms were blocked, death to the animal would ensue quickly.

Having established a link between hyperglycemia, enhanced activation of PKC-beta and vascular complications, Dr. King began working with researchers from Eli Lilly and Company to design a chemical inhibitor for the PKC-beta isoform. From this collaboration, a new drug, ruboxistaurin, emerged.

At the same time, the King lab set out to test their theory that activation of PKC-beta was involved in causing vascular complications. The team developed genetically altered mice that produced excessive amounts of PKC, tested an oral inhibitor to block PKC activity, and evaluated the effect of the PKC inhibitor on vascular dysfunction in the retina and kidney. The results, published in the journal *Science* in 1996, demonstrated for the first time in animal models that a PKC-beta inhibitor can correct some of the blood vessel abnormalities in the retina and the kidney associated with diabetes, and therefore abnormal activation of PKC-beta contributes to vascular complications. These studies, conducted with researchers from Eli Lilly, then entered clinical trials.

PKC and Diabetic Eye Disease

In other experiments using a PKC inhibitor, the King Lab sought to understand the relationship between VEGF and PKC in the development of diabetic eye disease. The major cause of blindness in people with diabetes is abnormalities of the tiny blood vessels of the retina. In some cases, the damaged vessels become more permeable, leaking fluid into the area of the eye responsible for sharp, central vision. In other cases, new blood vessels grow where they should not, causing bleeding into the eye and eventual loss of sight. This research, published in 1996, showed that the PKC pathway is at least partially involved in the action of VEGF, a substance that stimulates the formation of new blood vessels (neovascularization).

In collaboration with Lloyd P. Aiello, M.D., Ph.D., Head of Joslin's Section on Eye Research and Director of Joslin's Beetham Eye Institute, and Sven Bursell, Ph.D., an investigator in the section, Dr. King showed that injection of VEGF activates PKC in the retina. Findings of this study, published in the journal *Diabetes* in 1997, provide the first evidence that VEGF increases PKC *in vivo*, leading to greater permeability and neovascularization of the blood vessels of the eye. In addition, the PKC-beta inhibitor proved effective in treating these blood vessel abnormalities.

In 2002, Drs. King and Aiello again worked together in studies using transgenic mice overexpressing PKC-beta 2 and knockout mice not expressing any PKC-beta 2. From this work, published in the *Proceedings of the National Academy of Sciences* in 2002, the team demonstrated a dramatic increase in the development of new blood vessels in the overexpressors and a significant decrease in new blood vessels in the knockout mice, showing that PKC-beta is involved in angiogenesis in the retina.

Continuing PKC Research

In experiments using transgenic mice and PKC-beta inhibitors, the King lab showed that activation of the beta 2 isoform of PKC can cause specific changes leading to cardiomyopathy (disease of the heart muscle). In this research, published in 1997, transgenic mice overexpressing PKC-beta 2 in the myocardium (the middle layer of the heart wall) developed enlargement of the heart and other cardiac dysfunctions, which improved with treatment using the PKC inhibitor.

In the same year, the King lab studied the biochemical changes induced by high concentrations of glucose in cultured mesangial cells (specialized cells of the kidney) and in the glomeruli of diabetic animals. Researchers tested the PKC inhibitor in both models, demonstrating for the first time that the inhibitor can prevent chronic changes associated with hyperglycemia and diabetic kidney disease. The breakthrough results were published in the *Journal of Clinical Investigation* in 1997.

Further studies in 1999 demonstrated that PKC-beta and PKC-delta isoforms are activated by diabetes or elevated glucose. Research in both cultured cells and animal models, published in the journal *Circulation* in 2000, demonstrated that PKC is also involved in the activation of endothelial nitric oxide synthase, or eNOS, an enzyme in the lining of blood vessel walls that controls circulation, thus contributing to the dysfunction of vascular endothelia.

In 2002, collaboration with Gordon Weir, M.D., Head of Joslin's Section on Islet Transplantation and Cell Biology, suggested that some of the insulin abnormalities in pancreatic beta cells are mediated by PKC-beta 2, which regulates the expression of a

transcription factor, c-Myc, which in turn may be involved in the suppression of the insulin gene.

More recently, research in the King lab showed that PKC is involved in leukostasis (the abnormal clumping or adhesion of white cells to blood vessel walls, which decreases blood flow), and that treatment with a PKC inhibitor prevented adhesion and decreased oxidative stress (the accumulation of destructive molecules, such as free radicals). This evidence – the first of its kind – was published in the journal *Diabetes* in 2003.

Next Steps in PKC Research

Led by the efforts of Dr. King, researchers across Joslin Diabetes Center are committed to understanding the molecular mechanisms by which hyperglycemia damages vascular tissues and testing PKC inhibitors not only to advance scientific knowledge but also to prevent the long-term complications of diabetes.

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