Development of diabetic nephropathy: Involving factors

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Diabetes mellitus is a common disease worldwide. Diabetic patients with un-controlled hyperglycemia undergo diabetic complications including heart disease, kidney disease, retinopathy and neuropathy which are irreversible. High blood glucose concentration is the pivot cause of generalized pathophysiological changes. Autoxidation of glucose produces many free radicals which can directly damage the cells. Chronic diabetic patients with the renal complication always develop nephropathy and reach the end stage of renal disease. Accumulation of extracellular matrix materials such as collagen, fibronectin etc. which are induced by transforming growth factor- \( \beta \)1 (TGF-\( \beta \)1), causes the renal sclerosis. TGF-\( \beta \)1 is a key factor in the development of diabetic nephropathy. It stimulates the mesangial cells and endothelial cells of glomerular capillaries to produce collagen and other extracellular matrix materials. High concentrated glucose milieu induces an overproduction of TGF-\( \beta \)1 from mesangial cells in the glomeruli. The increase in the matrix materials results in renal vascular and tissue damage and loss of the renal functions. In addition, TGF-\( \beta \)1 could stimulate the mesangial cells to produce glucose transporter 1 (GLUT 1). The overproduction of GLUT 1 enhances the transportation of glucose into the mesangial cells resulting in the recurrence of those processes. Advanced glycated end-products (AGEs) are synthesized from the reaction between glucose and protein or lipid resulting in cell and tissue damages. Not only that AGEs are increased, but reactive oxygen species (ROS) is also generated during the synthesis of AGEs. In addition, diacylglycerol (DAG) is synthesized during the glucose metabolism. The increase in DAG, AGEs and ROS will activate protein kinase C (PKC) which is involved in the synthesis of many kinds of growth factors including TGF-\( \beta \)1 leading to nephropathy and other microvascular complications in

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diabetes mellitus. Sorbitol is also produced in polyol pathway in glucose metabolism. In diabetes, the accumulation of sorbitol in the eyes and kidneys causes changes in osmotic gradient that damage cells and tissues. The mechanisms of diabetic nephropathy development occur at the early stage of diabetes mellitus. Therefore, prevention and control of hyperglycemia are the prior essential treatment at the early diagnosis to inhibit key causal factors of diabetic nephropathy development. Early detection of excessive glucose in the body may be useful for those who tend to develop diabetes mellitus. The determination of glycosylated hemoglobin (HbA1c) level is a tool for early detection of excessive blood glucose.

Keywords: Diabetic nephropathy, Involving factors.

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Received for publication: July 5, 2007.

Objectives: 1. To study causal factors of diabetic nephropathy development.
2. To understand the mechanisms of the development of diabetic nephropathy.
มาเรียม อยู่สุขสวัสดิ์  การเลือกสภาพของโคเนื้อจากโรคร้าวผิว: ปัจจัยที่เกี่ยวข้อง  
จุฬาลงกรณ์มหาวิทยาลัย 2550 พ.ย. - ธ.ค; 51(11): 507 - 25

โรคราเราะยานเป็นโรคที่พบได้บ่อยและพบได้ทุกภูมิภาคของโลก ผู้ป่วยโรคราเราะพบเร็วกว่า ๆ มักจะมีอาการที่ซับซ้อนซึ่งเกี่ยวข้องกับระบบของตัวเลือดต่าง ๆ โดยเฉพาะอย่างยิ่งตัวเลือดแดงของโรค ทำให้ผู้ป่วยมีการต้านทานโรคได้อย่างมีประสิทธิภาพ และมีการปรับตัวต่าง ๆ ที่เกี่ยวข้องกับการเปลี่ยนแปลงโครงสร้างของปอดและลำไส้ ที่มีกลไกการยึดเหนี่ยวซึ่งสูง ๆ จะกระตุ้นให้เกิดปฏิกิริยาเข้มข้นมากยิ่งขึ้น และมีการสร้างสารบางชนิดที่ส่งผลต่อการเติบโตและข้อularity ที่มีการกระตุ้นให้ mesangial cell ในกลไกของโรคอาจสร้าง transforming growth factor-β1 (TGF-β1) เพื่อสร้างเมื่อกระตุ้นไม่เกิด mesangial cell และ endothelial cell ของหลอดเลือดใหญ่การสร้าง และสะสมสารพวก collagen เกิดการสูญเสียหลอดเลือดโดยกล้ามย้อย (glomerulosclerosis) และทำให้เกิดการเกิดการเกิดภาพของโคเนื้อเรื้อรัง (diabetic nephropathy) การเพิ่มขึ้นของ TGF-β1 กระตุ้นไม่เกิด mesangial cell ในการสร้าง glucose transporter 1 (GLUT 1) เพื่อส่งเรียม ซึ่งทำให้มีการขนย้ายโคเนื้อเข้า mesangial cell เพิ่มขึ้น แต่เสริมให้เกิดการปรับตัวและการเปลี่ยนแปลงต่าง ๆ เพิ่มขึ้นอย่างรวดเร็ว มีหลักฐานเย็นที่ TGF-β1 และ GLUT 1 อาจส่งเสริมการสร้างเชื้อปานที่เกิดกัน และแก้ นอกจากนี้การกระตุ้นของโคเนื้อในเส้นเลือดใหญ่จะทำให้มีการสร้างสารพวก advanced glycosylation end-products (AGES) ซึ่งเกิดจากการที่กล้ามย้อยส่งปฏิกิริยาภูมิคุ้มกันไปยังตัวเลือดใหญ่ ทำให้มีการเปลี่ยนแปลงของความขัดขืนและโครงสร้างของเนื้อเยื่อ ซึ่งทำให้เกิดการผิดปกติการ overwrite ในตัวเลือดใหญ่ที่เกิดขึ้นจะกระตุ้นไปยังการสร้างของ mesangial cell ในเส้นเลือดใหญ่ เพิ่มขึ้นอย่างรวดเร็ว การกระตุ้นของ AGEs อาจส่งเสริม reactive oxygen species ขึ้น ๆ ด้วย ทำให้เสริมการเกิดการผิดปกติของโคเนื้อ นอกจากรายนี้ในกระบวนการกลายกล้ามย้อยเกิดสาร diacylglycerol (DAG) เพิ่มมากขึ้น ทั้ง DAG, AGEs และอนุมูลวิสภาวะกระตุ้น protein kinase C ซึ่งเป็นเอนไซม์ที่กระตุ้นปฏิกิริยา phosphorylation ของกระบวนการซึ่งเนื้อเยื่อต่าง ๆ ในการสร้าง growth factors ต่าง ๆ รวมถึง TGF-β1 ซึ่งเป็นสารสำคัญที่ทำให้เกิด diabetic nephropathy และ microvascular complications และการกระตุ้นของโคเนื้อที่ยืดเข้าเนื้อเยื่อต่าง ๆ กระตุ้น polyol pathway ทำให้มีการสร้างและสะสมสารพวก sorbitol ในกล้ามย้อยและเพิ่มขึ้นและเกิดการต้านทานของเซลล์เนื้อเยื่อเหล่านี้
การเปลี่ยนแปลงต่าง ๆ ที่กล่าวมานี้เกิดขึ้นในระยะเริ่มแรกของการตรวจพบโรคเบาหวาน การมีระดับ
กลูโคสในเลือดสูงเป็นปัจจัยสำคัญของการเกิดภาวะอัลῆร่าทางของโรค ดังนั้นหากผู้ป่วยไม่ควบคุมระดับ
กลูโคสในเลือดที่สูงนั้นและทั้งไว้เร็วกว่า จะเป็น_CLICKED โดยมีการเปลี่ยนแปลงของไตและอวัยวะอื่น ๆ
ตามมา ซึ่งไม่สามารถจะรักษาให้กลับคืนสภาพเดิมได้ การตรวจหาระดับ glycosylated hemoglobin
(HbA1c) เป็นอีกวิธีหนึ่งที่สามารถบอกได้ว่าผู้ป่วยแตกต่างกรณีการมีกลูโคสเกินความจำเป็นแล้วและอาจ
พัฒนาไปสู่โรคเบาหวานได้หากไม่ควบคุมดังกล่าว ตรวจพบการเปลี่ยนแปลงต่าง ๆ ของโรค
เบาหวาน เช่น การใช้ PKC inhibitor ด้วยการแก้ปัญหาที่ปลายเหตุ ถือแม้ว่าจะเป็นสิ่งจำเป็นที่
ต้องกระทำก็ตาม การตรวจหักกลูโคสหมู่และมีการควบคุมอย่างเคร่งครัดเพื่อให้ระดับน้ำตาลในเลือด
ปกตินั้นเป็นสิ่งแรกที่ต้องคำนึงถึงและจัดการทันทีเมื่อตรวจพบ เพื่อป้องกันการเกิดการเปลี่ยนแปลง
ต่าง ๆ อย่างต่อเนื่องตามมาจนทำให้เกิดโรคไตอ่อนเป็นโรคแทรกซ้อนของผู้ป่วยเบาหวานที่ไม่สามารถ
แก้ไขให้กลับสู่สภาพเดิมได้อีกต่อไป

ค่าสำคัญ : การเปลี่ยนสภาพของไต โรคเบาหวาน ปัสสาวะที่เกี่ยวข้อง
Diabetes mellitus is a common disease worldwide. The prevalence of diabetes in the adult population is estimated at 5.1% in 2003, and tends to increase to 6.3% by 2025. Recently, the Southeast Asian region has 13.3% high prevalence of diabetes and impaired glucose tolerance; and it is expected to be the highest in the world by 2025. In Thailand, the estimated national prevalence of diabetes in Thai adults has been reported as 9.6% (2.4 million people). The most common diabetic complication is nephropathy. The prevalence of diabetic nephropathy in Thailand was about 43.8% of all diabetic complications.

Diabetic nephropathy is a renal complication that attacks a number of diabetic patients with uncontrolled hyperglycemia. Since it causes irreversible kidney failure leading to end-stage renal disease, it is a serious problem of diabetic patients. The approach to the causal factors and the mechanism of diabetic nephropathy development is helpful for those who are involved in the prevention and treatment of diabetic complications. High blood glucose concentration is the crucial cause of renal pathophysiological changes in diabetic patients. It induces oxidative stress and biochemistry disturbances that generate many causal factors of diabetic nephropathy including ROS, advanced glycated end-product (AGEs), diacyl glycerol (DAG), PKC, TGF-β1, polyol pathway (impair the ratio of NADH to NAD and accumulation of sorbitol in the renal glomeruli). Glucose transporter 1 (GLUT 1) is a factor which attributes to the development of diabetic nephropathy. This article review brings up some key causal factors of diabetic nephropathy development. However, other factors including angitensin II, molecular factors-induced growth factor synthesis, molecular binding are not stated in the comments.

In the beginning, this article reviews the roles of hyperglycemia, hypertension and proteinuria related to molecular mediators of the nephropathy development. The latter is focused on some biochemical factors and mechanisms involving in the development of diabetic nephropathy.

1. Hyperglycemia

Diabetes mellitus with poor blood glucose control contributes to the development of albuminuria. It has been elucidated that hyperglycemia is involved in morphological and functional abnormalities in diabetic kidney disease. Also, in extracellular ambient, glucose reacts non-enzymatically with primary amines of proteins, forming glycated compounds. When glucose is transported into cells by glucose transporters, it is partly metabolized to sorbitol via polyol pathway and then to hexosamines. All these biochemical pathways have been implicated in hyperglycaemia-induced kidney damage. Furthermore, excess glucose can directly exert toxic effects by activating intracellular signaling pathways and inducing a number of cytokines that injure the kidney.

1.1 Glucotoxicity

High glucose milieu has been confirmed that it directly alters the extracellular matrix deposition in the kidney. Some studies on the mesangial cells as well as tubular epithelial cells demonstrated that high glucose concentrations induce cellular hypertrophy and increase extracellular matrix components such as collagen, laminin and fibronectin. A further mechanism, whereby high glucose concentrations lead
to matrix deposition, is the reduction of the activity of metalloproteinases, the enzymes responsible for the extracellular matrix degradation. In the mesangial cells, high glucose levels induce transcription and secretion of TGF-β1.

Kidney cells do not have an absolute requirement of insulin for glucose uptake. Therefore, the intracellular glucose level more directly reflects its plasma concentration. The importance of excess glucose entry into the mesangial cells is an increase in extracellular matrix production and over-expressed cellular glucose transporter GLUT-1. The increase in GLUT-1 expression leads to the increase of basal glucose uptake, with consequential increase in aldose reductase expression and PKC activation. This results in the increase in extracellular matrix production. When antisense GLUT-1 is used to protect the mesangial cells from glucose-induced GLUT-1 over-expression, high glucose concentrations fail to induce extracellular matrix proteins. This finding indicates that the factors regulating glucose transporter expression or activity could influence glucose uptake and glucotoxicity. Glucose itself as well as TGF-β1 could up-regulate GLUT-1 expression in mesangial cell culture.

Glucose is metabolized to fructose-6-phosphate in glycolysis. Fructose-6-phosphate is converted to glucosamine-6-phosphate by a rate-limiting enzyme of glucosamine-fructose-6-phosphate amidotransferase in the hexosamine pathway. This leads to the formation of N-acetylglucosamine which is a component of membrane glycoproteins. Thus, the activation of the hexosamine pathway is implicated in the development of chronic diabetic complication associated with PKC activation and TGF-β1 over-expression.

1.2 Non-enzymatic glycation

Chronic hyperglycaemia leads to non-enzymatic protein glycation. The glycation results from exposure of lysine amino-terminal groups of proteins to high glucose concentrations. The increase in the covalent binding of glucose into proteins results in the formation of Schiff base which subsequently forms stable ketoamines, the Amadori products. When these glycated proteins undergo other reactions, such as dehydration, cyclization, oxidation and rearrangement, they form advanced glycation end-products (AGES). The reaction is not reversible, and AGES gradually accumulate in the tissues.

The accumulation of AGES in the kidney is parallel to the development of albuminuria, mesangial expansion and thickening of glomerular basement membrane in diabetes mellitus. The molecular cross-linking is also found in blood vessels that causes diabetic vascular complications. AGEs have various AGE-specific receptors. AGE-binding proteins or receptors for advanced glycosylation end-products (RAGE such as AGE-R1, AGE-R2, AGE-R3), lysozyme and macrophage scavenger receptors transduce the action of AGEs. AGE-specific receptors are present in many cell types, including the mesangial cells, glomerular epithelial cells and tubular epithelial cells. Interaction of AGE-modified proteins with AGE receptors results in the degradation of AGE proteins, and simultaneously induces the synthesis and release of cytokines, such as TGF-β1, platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF). Consequently, they result in the enhancement of collagen, laminin and fibronectin production.
1.3 The polyol pathway

Hyperglycemic condition increases the production of ROS which is implicated in sorbitol accumulation. Glucose is reduced to sorbitol by the enzyme aldose reductase in the polyol pathway. Excessive flux of glucose in the polyol pathway results in the increase in the ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD). This results in metabolic imbalances, mimicking the effects of the same redox change induced by hypoxia. In chronic diabetes, sorbitol accumulates in various tissues including the renal glomeruli and tubules. The accumulation of sorbitol disturbs the cellular osmoregulation by depletion of myoinositol and by changing in the cellular redox potential, resulting in permanent tissue damage in chronic diabetes mellitus. The polyol pathway is, therefore, involved in the pathogenesis of diabetic nephropathy and associated with GLUT 1, PKC activation and TGF-β1 production. Vascular endothelial cells are also affected by sorbitol accumulation causing vascular complication in diabetes mellitus.

2. Hypertension

Hypertension plays a critical role in the progression of diabetic nephropathy. The development of proteinuria mostly takes place parallel to the gradual rise in the systemic blood pressure. The increase in blood pressure is closely related to the speed of the decline in glomerular filtration rate. Therefore, diabetic patients with normal albumin excretion who have higher arterial blood pressure eventually progress to microalbuminuria. The elevated arterial blood pressure causes glomerular lesions. Antihypertensive therapy can prevent the occurrences of the proteinuria and the renal changes in diabetes mellitus.

3. Proteinuria

Proteinuria is a key feature of renal disease and a strong predictor of the progression toward end-stage renal failure. Proteinuria not only reflects renal impairment and a key pathogenic element of disease progression but also the advancement of generalized vascular damage. An excessive protein overload can induce tubulo-interstitial damage and contributes to the disease progression. Excessive tubular reabsorption of proteins and the consequent accumulation of proteins in tubular epithelial cells induce the release of vasoactive and inflammatory mediators, such as, TGF-β1 endothelin 1, osteopontin and macrophage chemotactic protein-1. These factors in turn lead to infiltration of mononuclear cells, causing injury to the tubulo-interstitium, and ultimately the renal damage. The changes in renal hemodynamics, either primary or reactionary to nephron loss, induce further proteinuria that contributes progressive renal impairment.

Microvascular complications in diabetes mellitus

Nitric oxide (NO) is a potent vasodilator molecule which is produced by the endothelial cells. Nitric oxide synthase (eNOS) catalyzes the reaction of L-arginine that changes to NO. NO inhibits the migration and proliferation of vascular smooth muscle cells. Furthermore, platelet aggregation and adhesive molecule expression of leukocyte are inhibited by NO. The injured vascular smooth muscle cells, endothelial cells, and the activated vascular wall mast cells, fibroblasts,
macrophages, and leukocytes, as well as the oxidation of norepinephrine (NE) from the renal sympathetic nerves produce abundance of ROS. ROS then interacts with NO to form the potent cytotoxic (OONO). Peroxynitrite radicals interact with proteins in the kidney leading to the glomerular and tubular dysfunction (Figure 1).

The causal factors of coronary artery disease and progressive renal insufficiency adversely affect endothelial cell function and vascular smooth muscle cell function. They induce the formation of reactive oxygen species such as superoxide anion and hydrogen peroxide. These ROS result in the decreases in the vasodilators and the growth inhibitors such as

**Afferent arteriole**

*Figure 1.* ROS reduces the biological effects of NO and induces the renal vascular complications.


**Figure 2.** Risk factors of the endothelial dysfunction lead to the coronary artery disease and renal pathophysiology.

prostacyclin and nitric oxide but the increases in the formation of the endothelium-derived vasoconstrictors and growth promoters such as angiotensin II, endothelin-1, and plasminogen activator inhibitor (PAI-1). These changes lead to vascular complications, especially the consequential pathophysiology of the kidneys (Figure 2).

The progressive injury of the coronary and the kidney are involved in the increase in apoptosis or programmed cell death which contributes to the remodeling of vascular wall and the activation of cell adhesion molecules. The adherence of both mononuclear and polymorphonuclear leukocytes to the vascular wall results in the infiltration and the deposition of oxidized lipids in the vessel wall. This consequently causes vasoconstriction, hypertrophy and hyperplasia of the vascular smooth muscle cells and thrombus formation.

Microvascular damage is a common underlying change of diabetic complications including diabetic peripheral neuropathy, retinopathy and nephropathy. Chronic diabetics with microvascular complications and poor treatments often undergo lower extremity amputation, blindness, and end-stage renal disease. All of these are irreversible. The studies in diabetic animals have demonstrated that an over-activation of PKC results in the microvascular damage. Protein kinase C (PKC) is an enzyme that acts as a signal transducer that involves in many biochemical processes. There are at least 12 isoforms of PKC that are located in various tissues throughout the body. Two of the isoforms, specifically β1 and β2, are located within the nerves, eyes, and kidneys and have been hypothesized to play major roles in both the development and progression of microvascular complications in

![Diagram showing the relationship of factors involved in microvascular damage in diabetes mellitus.](image-url)

Figure 3. The relationship of the factors involving in the microvascular damage in diabetes mellitus. (49)
diabetic patients. Hyperglycemia activates at least four metabolic pathways that contribute to diabetes-induced microvascular damage. In the normal physiological glucose concentrations, the activation of PKC occurs via cell signal transductions. It is associated with a complex sequence of biochemical interactions through G-protein coupled receptor-mediated pathway. DAG is an intermediate substance of glucose metabolism which is an important mediator to signal the activation of PKC. The activation of PKC accounts for the renal pathophysiological changes including an initial increase in the glomerular filtration rate, mesangial matrix expansion, glomerular capillary crowding, and glomerular occlusion. Other pathways, including glycation, polyol pathway and superoxide overproduction also play a role in diabetic microvascular complications. The relationship of these factors involving in microvascular damage that leads to the diabetic complications is shown in the following diagram.

**Relationship of hyperglycemia, oxidative stress, TGF-β1, PKC and GLUT 1 in the development of diabetic nephropathy**

Diabetic nephropathy is one of microvascular complications in diabetes mellitus which finally leads to end-stage renal failure. Its pathological features are thickening of the glomerular and tubular basement membrane. Glomerulosclerosis is a conspicuous morphological change in diabetes mellitus. Studies in streptozotocin-induced diabetic rats found the glycemic control is an important way in the prevention of the development of glomerulosclerosis. Poorly-controlled diabetic rats with constantly high plasma glucose level showed significant increase in basement membrane-like materials and mesangial cell mass in comparison with well-controlled diabetic rats. 

*Hyperglycemia generates diabetic nephropathy-inducing oxidative stress*

Under physiological conditions, glucose normally undergoes oxidation, the process through which protein-reactive ketoaldehyde, hydrogenperoxide and highly reactive oxidants are produced. In diabetes mellitus, the generation of reactive oxygen species is abundant. Hyperglycemia does not only generate more reactive oxygen species but also impairs antioxidant mechanisms leading to nephropathy in diabetes mellitus. Several *in vitro* studies have confirmed that highly concentrated glucose directly induces the increase in oxidative stress in glomerular mesangial cells. A study in kidney-cortex tubules of diabetic rabbits indicated an increase in intracellular hydroxyl free radical generation and change in intracellular glutathione status. The intracellular glutathione redox state was diminished (GSH/GSSG) in spite of the elevation of glutathione reductase activity. The activities of xanthine oxidase and catalase in the diabetic rat kidney were decreased. Insulin treatment could restore antioxidant enzyme activities. In addition, other antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase also decreased in diabetes mellitus. Advanced oxidative stress in uncontrolled diabetes manifested marked alterations in tissue antioxidant status. In addition, hyperlipidemia, which results from the chronic hyperglycemia, can induce the nephropathy via the generation of reactive oxygen species.
The attachment of these reactive oxygen species to proteins contributes to protein fragmentation and cross-linking in diabetic tissues.\textsuperscript{(14, 69, 70)} \textit{In vitro} and \textit{in vivo} studies indicated that lipid peroxidation also enhanced both diabetic animals and humans causing renal damages.\textsuperscript{(56, 61, 71)} Furthermore, an elevation of free radicals can induce apoptosis, which also contributes to the development of diabetic nephropathy.\textsuperscript{(8, 72, 73)} In addition, oxidative stress increases the production of TGF-β1 via the PKC activation.\textsuperscript{(5, 74)}

**Hyperglycemia stimulates TGF-β1 overproduction via PKC activation**

Transforming growth factor-beta 1 (TGF-β1) is found an important mediator in the development of diabetic renal disease.\textsuperscript{(7)} It stimulates the production and accumulation of glomerular matrix materials.\textsuperscript{(76, 77)} The urinary levels of TGF-β1 increase in diabetic patients.\textsuperscript{(78)} The neutralization of TGF-1 by anti-TGF-β1 antibody has been shown to attenuate renal hypertrophy and thus enhance extracellular matrix gene expression in STZ-induced diabetic mice.\textsuperscript{(9)} In addition, a long-term administration of neutralizing anti-TGF-β1 antibody could prevent glomerulosclerosis and renal insufficiency in diabetic db/db mice, a genetic model of non-insulin dependent diabetes mellitus.\textsuperscript{(77)} The stimulation of TGF-β1 expression with high glucose concentration milieu has been demonstrated in the culture of glomerular mesangial cells and proximal tubular cells.\textsuperscript{(7)} The high glucose concentration ambient and mechanical stretch could stimulate the production TGF-β1 via a PKC-dependent mechanism.\textsuperscript{(79, 80)} An \textit{in vitro} study demonstrated that highly concentrated glucose ambient could induce TGF-β1 over-expression and increase extracellular matrix protein in the renal glomeruli.\textsuperscript{(15, 81)} The highly concentrated glucose induces TGF-β1 gene expression via two adjacent activating protein-1 (AP-1) binding sites.\textsuperscript{(82)} It enhances the binding activity of the nuclear proteins to the AP-1 binding sites which results in the increase of activity of TGF-β1 promoter. Protein kinase C (PKC) and p38 mitogen-activated protein kinase (p38 MAPK), which regulate the TGF-β1 promoter activity, are also activated by the highly concentrated glucose.\textsuperscript{(83)} The events result in the over-expression of TGF-β1-mRNA and protein.

**TGF-β1 induces overproduction of extracellular matrix materials via PKC activation**

Under concentrated glucose conditions, the increased in \textit{de novo} synthesis of DAG from glucose has been demonstrated in the glomerular mesangial cells cultured and in the glomeruli of diabetic rats. The increased DAG activates PKC resulting in the activation of the various intracellular signal transduction systems.\textsuperscript{(79)} It has been shown that TGF-β1 over-expression stimulates PKC translocation and activation which accounts for the increase in the extracellular matrix materials.\textsuperscript{(80, 84)} The inhibition of PKC effectively blocks highly concentrated glucose-induced fibronectin production.\textsuperscript{(73)} A long-term administration of a PKC\textsubscript{β} inhibitor inhibits the glomerular PKC activation, reduces urinary albumin excretion rates and prevents the mesangial expansion in diabetic db/db mice.\textsuperscript{(80)} Inhibition of PKC\textsubscript{β} could decrease the basal and TGF-β1-stimulated collagen I production in human mesangial cells.\textsuperscript{(95)}
Highly concentrated glucose milieu induces GLUT 1 over-expression.

GLUT 1, which is a major glucose transporter of the mesangial cells, plays an important role as a glucose transporter in the pathogenesis of glomerulosclerosis. It is up-regulated in diabetic kidneys. An over-expression of GLUT1 could increase aldose reductase, a protein kinase Cα and the native GLUT1 transcription in both normal glucose and highly concentrated glucose condition in the mesangial cells. A study in a mesangial cell culture demonstrated that high concentrated glucose milieu (20 mM) facilitated the GLUT 1 expression in the rat mesangial cells and the uptake of glucose analog 3H 2-deoxyglucose (2H-DOG) when compared with mesangial cells in the physiological glucose concentration milieu (8 mM). The transport of glucose into the cells has been shown to be a rate-limiting for extracellular matrix production in the mesangial cells. A study in STZ-induced diabetic rats demonstrated that 45 days of diabetes resulted in an increase in albuminuria, urinary TGF-β1 and GLUT1 protein. The treatment of TGF-β can regulate GLUT1 mRNA and protein levels and significant increase in glucose uptake in rat mesangial cells. The cultured mesangial cells transduced with the human GLUT 1 gene and thus over-expressed the GLUT1 protein showed a marked increase in glucose uptake and synthesis of extracellular matrix molecules. The simultaneous presence of long-term high glucose concentration and TGF-β1 over-expression could enhance GLUT 1 up-regulation in the mesangial cells. With those evidences, hyperglycemia can induce the development of diabetic nephropathy via GLUT1 over-expression. The abundance of GLUT 1 results in the enhancement of glucose uptake and turns to stimulate TGF-β1 production in mesangial cells. Both proteins of TGF-β and GLUT 1 can influence the expression of one another.

This diabetic complication occurs in the first few years after diagnosis and could be detected early. Hyperglycemia is the most common cause of diabetic complications. Therefore, good control of blood glucose concentration is the first and pivot treatment in the prevention of diabetic complications such as nephropathy and other microvascular complications. In addition, a good management of hyperglycemia may delay the progression to the end stage renal disease. Early detection of excessive glucose in the body may be useful for those who tend to develop diabetes mellitus. The determination of glycosylated hemoglobin (HbA1c) level is a tool for early detection of excessive blood glucose.

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