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Pathophysiology of diabetic complications

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Dr. Helen Vlassara from New York, USA reviewed the chemical basis of advanced glycosylation end products (AGE) formed in excess in response to hyperglycaemia. Current data suggest that they are one of the major contributors to the pathogenesis of diabetic complications. Clinical trials with aminoguanidine, preventing AGE formation are ongoing, and recently cleavage of existing AGEs was demonstrated. Dr. Patrick Sharp from Harrow, UK reviewed the role of growth factors in diabetic retinopathy and emphasized data suggesting a prominent role for vascular endothelial growth factor (VEGF) in promoting angiogenesis and proliferative retinopathy.

Helen Vlassara: Glycation products and diabetes complications: molecular basis and therapy

Dr. Vlassara reviewed the importance and the broad role of non-enzymatic, hyperglycaemia-induced glycation on proteins and lipids as a causative factor for diabetes complications. Glycation leads to the production of reactive (glycotoxins) and non-reactive advanced glycation end products (AGE), the catabolic products of which are excreted in the urine. The level of AGE correlates with duration of diabetes and severity of complications, especially nephropathy. Of note, diabetic patients with end-stage renal disease who undergo haemodialysis exhibit high levels of AGE peptides in the circulation. AGE content of foods increase with cooking (not boiling/steaming), bringing to the body an additional load of AGE. Injection of AGE is shown to stimulate glomerular extracellular matrix production, and glomerular sclerosis in non-diabetic mice and rats. Moreover, AGE injections accelerate atherosclerosis. Smoking increases the levels of AGE in serum and further enhances the deleterious effects of smoking on the vascular tree. In addition, the increase in extracellular matrix production leads to an increase in the local availability of growth factors, resulting in albuminuria and sclerosis. On a broader scale, AGE are implicated in the ageing process.

Numerous AGE-associated cellular responses are attributed to AGE-specific cell-surface receptors including AGE-R1, AGE-R2, AGE-R3, which have all been cloned. They are present in numerous cell types, including haematopoietic, vascular, smooth muscle, mesangial, and neural cells. Current evidence also points to the existence of a heterogeneous and complex system of highly conserved AGE-binding and AGE-transporting molecules, which may contribute to AGE catabolism, transport across plasma membranes, or cell activation leading to tissue-specific secondary responses. Her findings also point to a direct link between AGE receptor expression, AGE accumulation and tissue damage. To address the problem of AGEs, various therapeutic approaches can be used. One rational solution is to inhibit AGE formation, such as with aminoguanidine. A broad range of complications is shown to be inhibited by aminoguanidine in experimental diabetic animal models. This drug has now progressed to the stage of clinical testing. In addition, agents which can cleave existing cross-linked AGE macromolecules in vitro may show promise in reversing tissue damage due to AGE. Smoking should be strongly discouraged. The role of dietary manipulation, aimed at reducing the food content of AGE should also be further evaluated before definitive recommendations can be made.

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Patrick Sharp: Growth factors and diabetic retinopathy

Diabetic retinopathy is a prominent complication that virtually all diabetic patients will develop with time. Dr. Sharp reviewed the funduscopic appearance of various stages of retinopathy and the underlying pathophysiology. He pointed out that although photocoagulation (laser therapy) is effective and widely used, the exact mechanisms by which it works are poorly understood. One hypothesis is that laser therapy reduces the amount of angiogenic and other growth factors produced by the ischaemic retina. Current data suggest that VEGF is an angiogenic factor with a major role in the pathogenesis of proliferative retinopathy and the development of neovascularization. The mechanisms of VEGF action and its link with protein kinase C (PKC)(an enzyme involved in signal transduction of many growth factors/hormones) activation were reviewed by Dr. King in another session. Hyperglycaemia is known to upregulate protein kinase C because of the de novo synthesis of diacylglycerol. Specific PKC inhibitors (e.g. LY333531) have been studied in diabetic rats and shown to ameliorate proliferative retinopathy, but the safety of inhibiting this enzyme throughout the body is still an open question. Further research on the role of growth factors in diabetic retinopathy is indicated and may provide us with future therapeutic modalities.

Fuad Ziyadeh: Role of transforming growth factor beta (TGF-β) in diabetic renal hypertrophy and extracellular matrix expansion

Diabetes is associated with early glomerular and tubular hypertrophy and, as emphasized in the session on diabetic nephropathy, with extracellular matrix accumulation, eventually resulting in clinical manifest renal disease. The most abundant constituent accumulating in the renal extracellular matrices is type IV collagen, and its accumulation may be due in part to increased production and in part to reduced degradation. Intrarenal cytokines and growth factors are important in the regulation of these processes; in particular TGF-β stimulates the synthesis and inhibits the degradation of several classes of extracellular matrix molecules, and promotes cell hypertrophy. Thus this growth factor may be involved in the pathogenesis and progression of diabetic nephropathy.

In vitro studies. High glucose concentration in the culture medium stimulates both in mesangial and proximal tubule cells, the biosynthesis of collagen, and other extracellular matrix constituents, and modulates cell growth and hypertrophy. Also mRNA for TGF-β1 and the protein bioactivity are increased in high glucose medium. Interestingly incubation with anti-TGF-β antibodies results in reversal of the effects of high glucose on cell growth and prevents the stimulation of collagen mRNA expression and synthesis.

In vivo studies. TGF-β is overexpressed in the glomeruli and tubules of human and experimental models of insulin-dependent diabetes. The spontaneous development of diabetes in the BB rat and in the NOD mouse is associated, shortly after the appearance of hyperglycaemia and in association with the development of renal hypertrophy, with increased expression of TGF-β in the kidney. Also in the streptozotocin-diabetic mouse there is early stimulation of TGF-β and up-regulation of type II TGF-β receptor in the renal cortex. In this model the administration of a neutralizing antibody against TGF-β for 9 days was able to prevent glomerular hypertrophy and to reduce the increment in kidney weight without having any effect on blood glucose. This treatment also prevented the increase in mRNA encoding for a1(IV) collagen and fibronectin. These results indicate that in the diabetic mouse kidney hypertrophy and disturbances in extracellular matrix turnover are associated with up-regulation of TGF-β1 and that these early abnormalities can be prevented by treatment with neutralizing anti-TGF-β antibodies.

Recent studies performed in diabetic patients with various degrees of nephropathy found that TGF-β protein and mRNA levels were associated with the degree of diabetic glomerulopathy and of interstitial lesions. However TGF-β mRNA was also correlated with HbA_{1c} levels that were higher in patients with the most severe renal lesions. Also mRNA for TGF-β was increased in glomeruli of diabetic patients, but similar results were also found in several other glomerular diseases. These studies suggest that TGF-β may represent a common mediator for different progressive renal diseases, including diabetic nephropathy; however the signals involved in TGF-β stimulation are distinct in the different diseases and are worthy of further investigation.

In patients with NIDDM selective renal artery and vein sampling was performed and an enhanced net renal production of TGF-β1, associated with increased urinary excretion of TGF-β, has been reported, whereas non-diabetic subjects exhibited net renal extraction. These preliminary studies suggest a role for TGF-β in human diabetic nephropathy and further research is warranted.

Future directions and recommendations

- The prospect of effectively minimizing advanced glycation end products (AGE) formation may prove to be a breakthrough in the treatment of diabetic complications.

- Elucidation of the molecular events leading to the cellular toxicity of high glucose levels remains crucial and will constitute the basis for developing future therapeutic modalities. This point was discussed, not only in this session, but in all the sessions dedicated to complications of diabetes. Topics included the sorbitol pathway, non-enzymatic glycation, de novo synthesis of diacylglycerol, reductive and oxidative stress, the glucosamine pathway and altered calcium homeostasis. Nonetheless achieving optimal metabolic control cannot be overemphasized.
- Studies of factors shifting the balance of extracellular matrix production and degradation towards accumulation are central to the understanding of the pathogenesis and offer promise of specific interventions in diabetic nephropathy.
- In vitro studies and in vivo animal studies should then be the basis for human studies.
- Clinical trials with various therapeutic agents should be strongly encouraged, and preferably performed as a collaborative effort.