Cardiovascular complications of diabetes

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Cardiovascular complications are the leading causes of mortality in both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). The severity of this problem is clearly illustrated by the finding that IDDM patients suffer from mortality rates many times higher than of the general population [1]. In spite of the gravity of this complication, there is a relative paucity of data specifically on the pathogenesis, prevention and the treatment of the cardiovascular lesions in diabetes. The goal of this article is to highlight certain areas which are pertinent to diabetic cardiovascular complications and to suggest research goals which will bring understanding of the pathogenesis of this grave complication and its potential treatments.

George L. King: Hyperglycaemia and insulin resistance: how do they increase cardiovascular risk in diabetic patients?

A major cause of morbidity and mortality in diabetic patients is macrovascular disease affecting the heart and large vessels. In diabetes there is an acceleration of atherosclerosis with excessive extracellular matrix thickening. In the heart, the major pathology is atherosclerosis of the coronary arteries, and the fibrosis, and hypertrophy of the myocardium. Hyperglycaemia, hyperlipidaemia and insulin resistance are some of the recognized risk factors [2, 3]. The basic abnormalities are in glucose metabolism and insulin

action which can lead to a whole range of vascular changes in the areas of coagulation, contractility, leukocyte adhesions, and smooth muscle cell proliferation. The mechanisms by which hyperglycaemia causes vascular dysfunction are probably multiple. These mechanisms include non-enzymatic glycation, oxidative stress, polyol-myoinositol alteration, and activation of diacylglycerol (DAG) and protein kinase C (PKC) pathways. The role of the DAG and PKC pathways which regulate many vascular functions were discussed since this is the newest of the four main theories.

Studies over the last 7 years have shown that there is an increase in DAG levels and activation of PKC activities in vascular cells from the retina, glomeruli, aorta and the heart when exposed to high glucose levels in culture or in the diabetic state in vivo [4]. Increased glycolysis can elevate DAG which in turn activates protein kinase C in the vascular cells. The β I and II isoforms of PKC are predominantly increased in the vascular cells induced by diabetes and hyperglycaemia involving PKC β I in the renal glomeruli and PKC β II in the rest of the vasculature.

The increase in DAG levels and activation of PKC β isoforms have been associated with a variety of biochemical, cellular and physiological changes in the vasculature. These changes lead to an increase in the transcription rate of transforming growth factor β (TGF β) which regulates increases in the synthesis of extra-cellular matrix protein such as type IV and VI collagen, and fibronectin resulting in capillary basement membrane thickening which is observed in all diabetic animals and patients [5]. The abnormal amount of basement membrane matrix can lead to vascular dysfunction as well as acceleration of atherosclerosis. Other changes that are due to glucose induced activation of cPLA2, inhibition of Na⁺K⁺ ATPase which could be involved in the regulation of vascular permeability, regulation of caldesmone and

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plasma activator inhibitor (PAI)-1 expression. Increases in caldesmone and PAI-1 could be responsible for abnormalities of coagulation and contractility also reported in diabetic patients.

Another common problem observed in diabetic patients is cardiomyopathy. Up to 25% of diabetic patients with congestive cardiac failure do not have significant angiographic evidence of coronary vessel disease and there is generally a greater prevalence of congestive heart failure in diabetic compared with non-diabetic patients [6, 7]. This has been investigated using a transgenic mouse model which overexpresses PKC β isoform in the heart using a myosin heavy chain promoter to target the transgene to the myocardium. The transgenic mice develop enlarged hearts with fibrosis and necrosis leading to cardiac failure with congestion of the lungs and liver. These findings have clearly shown that a PKC abnormality as observed in the heart of diabetic animals can lead to cardiac failure and may be partially responsible for the cardiovascular abnormalities in diabetes.

Epidemiological studies have suggested an association among insulin resistance, hypertension and cardiovascular disease. A new theory is postulated suggesting that insulin's activities on the vasculature can be divided into atherogenic and anti-atherogenic actions [7]. The anti-atherogenic actions include vasodilatation and release of nitric oxide (NO), inhibition of tumour necrosis factor (TNF) and angiotensin II actions, amino acid transport and conversion of glucose into glycogen. However, in diseases with insulin resistance or insulin deficiency, these anti-atherogenic actions can be lost. In contrast, insulin also has atherogenic actions, especially in the hyperinsulinaemic state, such as increased basement matrix synthesis, smooth muscle cell and growth, PAI-I expression. At the molecular level, the anti-atherogenic actions could be mediated through insulin-receptor substrate 1 (IRS) 1 and 2 with the subsequent activation of phosphoinositol (PI)3 kinase. This pathway can be activated by insulin at low concentrations and can lead to anti-atherogenic actions such as NO production. However, this pathway is decreased or blocked in the insulin resistant state. Insulin can also signal through the activation of mitogen activated protein (MAP) kinase, which does not require IRS-1 or IRS-2 tyrosine phosphorylation which can lead to the cellular growth and PAI production which can, in turn, lead to an increase in coagulation and atherosclerosis. This new hypothesis of selective insulin resistance on the vasculature could prove to be useful in the design of new therapeutic agents to prevent cardiovascular complications of diabetes.

In summary, these theories provide an explanation at the molecular level for the biochemical and physiological changes relevant to the cardiovascular complications of diabetes. New treatments are being designed to test these hypotheses.

Philippe Froguel: Genetics of macrovascular disease

Many different mechanisms are involved in the development of macrovascular complications, e.g. glycation of proteins, oxidation of lipoproteins, alteration in lipoprotein metabolism, and cellular proliferation. None of these are specific to diabetes. No single gene has been identified relating to macrovascular complications. This suggests that genetic factors, if present, are randomly distributed in the population. Certain genes could be risk factors in the general population and the risk magnified in the presence of diabetes. It is difficult to find evidence for macrovascular genes in the general population, but the fact that the risk is higher in the diabetic population may allow easier identification in this group.

A mutation in lipoprotein lipase (LPL) has been identified in a family and found to cause increased levels of triglycerides and pancreatitis. If an individual is heterozygous for the mutation, they will probably have the same phenotype as the parent. However, if an individual inherits the mutation and, in addition, has hyperglycaemia, this gives a phenotype as if two copies of the gene were present [9]. A common allele of LPL has been found in 45% of the population. If individuals with the polymorphism are compared with siblings who do not have the allele, up to 8% difference in triglyceride level may be found. In contrast, if two diabetic siblings are compared, one who has the LPL variant with a diabetic sibling who does not, the triglyceride levels may be increased by 40–50%.

It is clear that many genes are involved. In a monogenic disease, a rare variant may have major defects, but this has a marginal effect for the population in general. However, in polygenic diseases, multiple frequently found gene variants may have minor effects separately. These genes may not be important for individual subjects, but rather more important for the population.

Genetic associations have been identified using a candidate gene approach, which is based on associations with pathophysiology. Intermediate phenotypes are identified. A number of strategies such as case control, family studies and prospective studies can be performed. Quantitative trait linkage analysis may be performed in affected sib pairs.

In a case-control study of coronary artery disease in NIDDM in a French Caucasoid population, with cases designated as having significant coronary artery disease (CHD) having had a myocardial infarction, and control subjects designated as having no clinical CHD and a normal ECG, a number of candidate genes were studied. A polymorphism in the angiotensin converting enzyme (ACE) gene was previously identified in France and Northern Ireland with three genotypes (DD, DI and II). An association was found between the DD genotype and myocardial infarction.

It was found that individuals with the DD genotype had higher ACE levels than those with the II genotype. Patients with NIDDM and CHD were found to have a D allele more often and an association was found with this allele and CHD in NIDDM. However, although the presence of a D allele was identified as a risk factor for CHD, the risk was not particularly high. The odds ratio was 1.44 for a DI genotype and 2 for a DD genotype which was no greater than the increased risk in individuals who smoked. It was concluded that the presence of a D allele is a risk factor for CHD in the general population and the risk is probably more important in hyperglycaemic subjects [10].

The other gene studied, apoprotein K or paroxanase (PON), is an enzyme bound to HDL. The activity is decreased in subjects who have had a myocardial infarction. Two gene polymorphisms have been identified. There is an increase in the B allele and BB genotype in NIDDM patients compared with control subjects. The presence of this allele gives an odds ratio of 1.91. PON may protect lipids against oxidation. In the general population, whether an individual possesses the A or B allele is probably not important, but in the presence of hyperglycaemia, oxidative stresses may be greater, and the presence of a genotype which encodes an enzyme with a lower activity may become important [11].

Finally, in a study of patients with nephropathy, a polymorphism (Ala/Thr 789) has been identified in the Von Willebrand factor, important in haemostasis. No association has been found between this factor and diabetic nephropathy. However, of 50 patients identified with CHD, Thr/Thr 789 appeared to be more frequently found in patients who did not have CHD. Ala/Ala 789 was more frequent in those who had CHD. A difference in the level of Von Willebrand factor was found dependent on whether the individuals had Ala/Ala 789, or Thr/Thr 789. It is possible that this may contribute to macrovascular disease in patients with IDDM.

At present, no major genes have been identified which have a bearing on macrovascular complications, using a candidate gene approach. Ultimately, there will be a need to search for genes using a positional candidate gene strategy. It is likely that a gene(s) will be identified which is unique for diabetic macrovascular disease. However, identification of genes in the general population will undoubtedly be of equal importance in diabetes.

Eberhard Standl: Influence of diabetes mellitus on the heart and macrovascular mortality

Dr. Standl from Germany gave an interesting lecture on the influence of diabetes on the heart and macrovascular mortality. He first raised the issue of risk

factors for macrovascular diseases in particular hypertension and microproteinuria which are greater in patients with NIDDM than IDDM. Not known however, is whether microalbuminuria is also a predictive factor of macrovascular complications. Among other risk factors he mentioned hypertension, dyslipidaemia with high LDL, low HDL-cholesterol and increased small LDL-cholesterol, the insulin resistance syndrome and hyperinsulinaemia. He reviewed the Care Study as an intervention study aimed at reducing cholesterol levels with pravastatin in both patients with (cholesterol < 250 mg/dl) or without diabetes. The number of coronary events was significantly reduced by the introduction of pravastatin in both groups; however, the effects were more pronounced in the non-diabetic group. This would suggest that elevated cholesterol may have a more pronounced effect in diabetic patients than in hyperlipidaemic patients alone. He then presented a 10-year follow up study of the Munich General Practitioner Project to evaluate the long term effects of the above mentioned cardiovascular risk factors in a random cohort of 290 patients with NIDDM. In an analysis, patients especially those who died from macrovascular causes had significantly high fasting blood glucose, HbA_{1c}, Von Willebrand factor protein, urine albumin excretion and serum B₂-microglobulin and exhibited significantly more ischaemic heart disease, carotid artery and peripheral vascular disease determined by ultrasound-Doppler. Given a multiple logistic analysis, the risk factors for macrovascular death were age, HbA_{1c} and Von Willebrand factor protein. When baseline macrovascular disease was taken into account, carotid artery disease was also a determinant. This study indicated that age and HbA_{1c} are major determinants, and that in NIDDM, Von Willebrand associated endothelial damage is a risk factor for macrovascular mortality. Interestingly, Dr. Standl mentioned that recent studies showed a close correlation between Von Willebrand factor protein and vascular endothelial growth factor (VEGF) and also insulin resistance. To assess cardiac autonomic neuropathy, Dr. Standl used the innovative technique of scintigraphic OJ/I × 2 detection with ¹²³metaiodobenzylguanidine (¹²³MIBG) in newly diagnosed patients with more prolonged IDDM. He found signs of sympathetic dysinnervation especially in the posterior myocardial region in 77 % of newly diagnosed IDDM patients, whereas, only 3 % of these patients showed all ECG-based cardiac autonomic neuropathy [12]. Of note, glycaemic control was able to restore this partial impairment. Thus, this study suggests that even in the early stage of IDDM, cardiac sympathetic denervation is composed of reversible and irreversible neuronal activities. On the other hand, in IDDM patients with longer duration of disease, sympathetic denervation was found independent of current HbA_{1c} levels. Dr. Standl also

presented data from the Shep Study showing a marked percentage reduction in stroke and myocardial events when hypertension was treated with beta blockers in NIDDM patients [13]. In NIDDM almost 50 % of patients had significant artery disease which is easily detected by ultrasound Doppler and ECG. Intervention studies using diet and exercise in subjects with the metabolic syndrome were able to reduce the risk of coronary artery disease, this was even more striking in individuals with higher body mass index.

Ross Gerrity: A model of accelerated atherosclerosis in diabetic swine

Ross Gerrity presented a new model of accelerated atherosclerosis which has been developed in swine. This is the only diabetic model which closely resembles human atherosclerotic disease. By feeding pigs a diet rich in cholesterol and lard, he was able to increase blood cholesterol to levels ranging from 300 to 700 mg/dl. These animals develop the classic lesions normally seen in humans; namely, initial fatty streaks with subsequent formation of advanced and complicated fibrosis plaques within 20–30 weeks of hyperlipidaemia in the aorta. Coronary artery lesions developed later (40–50 weeks) [14].

To study the development of atherosclerosis in the presence of diabetes, Dr. Gerrity induced diabetes by giving the pigs streptozotocin, which led to a nearly 90 % destruction of pancreatic beta cells. Mean blood glucose levels were approximately 16–17 mmol/l and glycosylated haemoglobin was clearly elevated. The pigs were not treated with insulin and remained in poor glycaemic control. Four groups of pigs were studied: Group 1 was normolipaemic and normoglycaemic. The second group was hyperglycaemic but normolipaemic; the third group was hyperlipaemic but normoglycaemic; while the fourth group was both hyperglycaemic and hyperlipaemic. Plasma cholesterol levels were approximately 400 to 600 mg/dl in both non-diabetic and diabetic hyperlipaemic animals. The animals that had not received the cholesterol-rich diet maintained cholesterol levels at 70–120 mg/dl. Plasma lipid and lipoprotein levels in the two groups that received the high cholesterol diet were not significantly different; the only major difference was significantly greater elevation of triglyceride levels in diabetic patients. This is a finding of considerable significance in that it mimics the hypertriglyceridaemia common in human diabetic patients. It is clear that it is associated with the diabetic state in this swine model, since hyperlipaemic, non-diabetic animals do not show elevated triglycerides. To determine the extent and progression of atherosclerosis, image analysis was performed on Sudan IV-stained lesions of the aorta. These animals were killed after

8, 12, 16, 24, 32, and 48 weeks. No lesions were found in the group with diabetes alone but lesions were found in the non-diabetic hyperlipaemic animals and in the diabetic hyperlipaemic group. However, atherosclerosis was greatly accelerated by diabetes in that the percent of aortic surface area covered with lesions was twofold greater in the diabetic hyperlipaemic group compared to hyperlipaemia alone, even as early as 8–12 weeks. Not only was the percentage of aortic area covered by lesions greater, but also coronary artery lesions were more advanced and more occlusive in diabetic animals. Necrotic changes were seen as early as 16 weeks. Typically, significant coronary atherosclerotic lesions in non-diabetic hyperlipaemic swine took 40 to 50 weeks to develop as opposed to the early (20 weeks) development that occurred in the diabetic hyperlipaemic animals. Severe atherosclerosis was found in these animals, with 85–95 % occlusion of coronary arteries and extensive calcification, neither of which was seen in animals with hyperlipaemia alone at 20 weeks. The cholesterol content of lesions was not different between hyperlipaemic and hyperlipaemic diabetic animals, but the esterification of cholesterol was doubled in diabetic lesions compared to animals with hyperlipaemia alone. The higher rates of cholesterol esterification may provide a clue to the acceleration of atherosclerosis in the presence of diabetes. Interestingly, the diabetic animals also demonstrated incipient glomerulosclerosis.

This new very humanoid animal model will allow detailed investigation of the mechanisms involved in the development of accelerated atherosclerosis in diabetes. It should also provide an invaluable tool to test new therapeutic strategies and to examine other complications of diabetes.

Future directions and recommendations

- Characterization of gene expression in atherosclerotic plaques obtained from diabetic animals and patients.
- Identification of endothelial or atherosclerotic markers in diabetes and their correlation with atherosclerosis in diabetes.
- Characterization of insulin actions in the vascular cells at the molecular and physiological levels.
- Study of the unique entity of diabetic cardiomyopathy – clinical course and characterization of genes which may contribute to its development in diabetic animals and patients.
- Design specific interventions to prevent coronary vascular disease in both IDDM and NIDDM patients: specific lifestyle changes and drugs to accomplish this.
- Identification of patients with IDDM at risk of coronary vascular disease – a need to begin to target

patients with IDDM at the time of development of microalbuminuria as the major cause of death is from ischaemic heart disease.

- Multinational collaboration on identification of genes involved in macrovascular disease in diabetes.
- Study gene expression induced by diabetes or hyperglycaemia.
- Study of accelerated atherosclerosis in IDDM.
- Identification of causes of sudden unexpected death.
- The use of the diabetic fat fed pig as a model for atherosclerosis should be further explored.

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