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Loss of beta-cells with fibrotic islet destruction in type 2 diabetes mellitus

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1. ABSTRACT

Recent morphologic analyses of human pancreases strongly suggest that a decreased beta-cell mass is observed from the early stages of diabetes and is caused by accelerated apoptosis of the beta-cells. In this article, we propose that fibrotic islet destruction might be one of the important pathogenic mechanisms of the limited capacity of beta-cell proliferation and accelerated apoptosis in diabetic patients. We have found that pancreatic stellate cells (PSCs) are involved in the progression of islet fibrosis in type 2 diabetes. High concentrations of glucose and insulin in islets contribute to PSC activation and proliferation through angiotensin II type 2 (ATII) signaling pathway, although the exact mechanisms remain to be confirmed. Angiotensin-converting enzyme inhibitors attenuate fibrotic islet destructions and that these have some beneficial effects on glucose tolerance. We suggest that PSCs might play a major role for the fibrotic islet destruction in patients with type 2 diabetes, and suppression of PSCs activation and proliferation might be one of the reasonable target to prevent and delay the progression of the type 2 diabetes mellitus.

2. INTRODUCTION

The number of people with type 2 diabetes mellitus has increased throughout the world, and the rate of increase shows no signs of slowing. The Diabetes Atlas estimates that there were 194 million people with diabetes in 2003, and predicts the number to increase to 333 million by 2025 (1), and arguably, the Asia–Pacific region is emerging as the epicenter of this epidemic (2). The increase in type 2 diabetes in Asia differs from that observed in other regions of the world in that it has been telescoped into a much shorter period. Furthermore, it develops in relatively young people and tends to occur at a much lower body mass index (BMI) (3, 4). Evidence exists that epidemics of type 2 diabetes can occur at different stages of urbanization; however, this evidence does not account for all the observed characteristics of epidemics. The most important factor predisposing to type 2 diabetes mellitus in this group is prominent early insulin secretory defects, which have been reported in the various countries within Asia (5, 6). Impaired insulin secretion might be induced by insufficient beta-cell mass, by functional defects within the beta-cells themselves, or both. Although a good linear

correlation between beta-cell mass and BMI has been reported in normal and type 2 diabetic patients, measured beta-cell masses in nonobese diabetic patients were lower than those in normal subjects and were not related to the duration of diabetes or to the glycosylated hemoglobin (HbA1c) levels of patients (7). Other investigators have also reported similar findings (8, 9). These results suggest that maximal beta-cell mass and the regenerative capacity of beta-cells in each patient's response to insulin resistance could be established at an early stage of life, either in the intrauterine environment or by genetically determined factors, or both. Furthermore, metabolic alterations related to diabetes also induce accelerated beta-cells loss and fibrotic islet destruction during the development and progression of the disease. However, we still do not clearly understand the major determinants of beta-cells mass in humans.

Beta-cells mass should be dynamically adjusted by external demand during adult life and is determined by the balance of neogenesis and self-replication and apoptosis of the beta-cells (10–14). In patients with diabetes, beta-cells apoptosis is thought to be significantly accelerated by various causes. Glucolipotoxicity (15), low-grade chronic inflammation (16), amylin deposition with fibrotic islet destruction (9, 16–20), and oxidative stress (21) might be the possible pathogenic causes of type 2 diabetes. The pathologic manifestations of the pancreatic islets of type 2 diabetic patients include a markedly reduced beta-cells mass, hyaline material deposition, and eventual fibrotic destruction of islet. These pathologic changes are commonly observed in humans and in several animal models of type 2 diabetes. It is not known whether islet destruction is a cause or a result of the pathogenic mechanisms of type 2 diabetes mellitus, but the resultant architectural distortion of pancreatic islets would be an important factor for development and progression of the disease and so it might be the important potential target for treatment of type 2 diabetes. Among the possible mechanisms, glucolipotoxicity-induced beta-cell dysfunction and accompanying beta-cells loss have been quite well studied; however, the role and pathogenesis of fibrotic islet destruction on the progression and development of type 2 diabetes mellitus have not yet been well studied. In this review, we provide an overview of the characteristic features and underlying pathogenesis of insulin secretory defects in patients with type 2 diabetes mellitus, especially those in Asia, and then describe the morphologic characteristics of the pancreatic islets. Finally, we would like to focus on pathogenic mechanisms and the possible clinical implications of preventing fibrotic islet destruction for the prevention and delay of the progression of the disease.

3. INSULIN RESISTANCE AND INSULIN SECRETION

3.1. Prominent insulin secretory defects in non-obese type 2 diabetic patients in Asia

Type 2 diabetes mellitus occurs when there is inadequate insulin secretion to meet the insulin demands of the body (22, 23). In many Asian countries, the majority of type 2 diabetic patients are nonobese, which is clearly in contrast to Caucasians. There appears to be an ethnic difference underlying the pathogenesis of type 2 diabetes mellitus, because the degree of obesity is closely related to insulin resistance. Haffner *et al.* reported that 92% of patients in the white population with type 2 diabetes mellitus were insulin resistant (24). However, a Japanese group showed that only 40% of their patients with type 2 diabetes mellitus could be subclassified into an insulin resistance group using homeostasis model assessments (HOMA) (25, 26). In Korea, the prevalence of diabetes mellitus is 8% to 10% and more than 95% of diabetic patients belong to the type 2 diabetes mellitus group. However, patients with a body mass index (BMI) greater than 25 kg/m^2 comprise only 35% (27-29). This suggests that the characteristics of Korean type 2 diabetic patients are quite different from those of type 2 diabetic patients in Western countries, but are similar to those in Japan and other Asian countries.

To determine the cutoff value for insulin resistance by HOMA in the general population in Korea, we examined 1,901 normal subjects aged 25 to 80 years. The mean HOMA-IR value (mean ± SD) of 1,901 normal subjects in Korea was 1.3 ± 0.8. Therefore, those patients with values greater than 2.5 (mean ± 1.5 SD) were defined as being insulin resistant (30). Patients with HOMA-IR values greater than 2.5 were defined as being insulin resistant according to our data, which was the same cutoff point defined in the Japanese study (31). Based on those results, we analyzed 267 Korean nonobese (BMI: ≤ 25 kg/m^2) patients with type 2 diabetes mellitus. The HOMA–IR values in the patients with insulin resistance and those with normal insulin sensitivity were 4.2 ± 1.4 and 1.5 ± 0.6, respectively. The percentage of insulin-resistant diabetic patients in our study was 23.6% (30) which is similar to data reported by Taniguchi *et al.* (31). In both studies, the prevalence of insulin-resistant variant was small, but this group was associated with a higher BMI and triglyceride (TG) level, and lower high-lipoprotein-cholesterol (HDL-C) level in the patients with the insulin-sensitive variant. In addition, a greater proportion of our type 2 diabetic subjects were insulin sensitive, had a lower BMI, and were characterized by a lower HOMA beta-cell value, indicating lower insulin output.

3.2. Early-phase insulin secretory defect may be the initial abnormality in the development of non-obese type 2 diabetes

Kim *et al.* have suggested that an early-phase insulin secretory defect was the sole determinant of the worsening of glucose tolerance in Korean subjects from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) without significant differences in insulin resistance, independent of the degree of obesity (32). In this study, there was no significant difference in insulin resistance as assessed by HOMA (R) among subjects with normal fasting glucose (NFG)/NGT, impaired fasting glucose (IFG)/NGT, and NFG/IGT. However, early-phase insulin secretion as assessed by the insulinogenic index was significantly decreased in subjects with IFG/NGT or NFG/IGT compared with those with NFG/NGT. Those

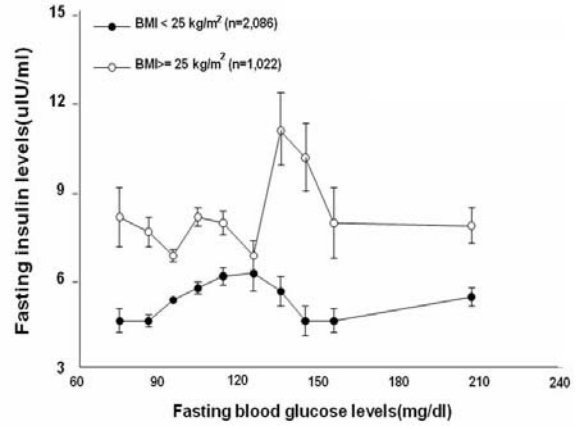

Figure 1. Fasting serum insulin levels according to the fasting blood glucose concentration in obese and nonobese normal, IGT, and diabetic subjects in Korea.

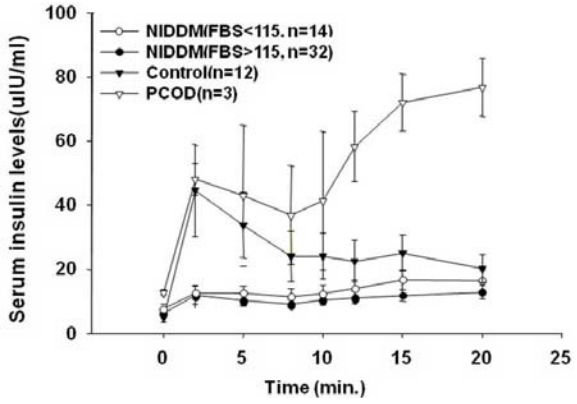

Figure 2. Acute insulin response to an intravenous glucose load over 20 minutes in normal, polycystic ovarian syndrome (PCOS), and diabetic subjects subdivided by fasting glucose concentrations after short-term intensive management of hyperglycemia.

results strongly suggest that an early-phase insulin secretory defect may be the initial event in the development of type 2 diabetes mellitus.

A Japanese group has also reported similar results. Interestingly, they compared the insulin response to oral glucose administration in Caucasian and Japanese people. In the Botnia study, using HOMA–IR, insulin resistance increased nearly twofold as glucose tolerance deteriorated from NGT to IGT, and to 3.6-fold in type 2 diabetes mellitus. The degree of this increment is remarkably higher in the Botnia study than in Japanese subjects. On the other hand, the insulinogenic index (30 min) of the Japanese subjects was low throughout the development of glucose intolerance from NGT via IGT to type 2 diabetes mellitus. In the Botnia study, the insulinogenic index was higher during all stages of glucose tolerance. It could be concluded that decreased insulin secretory capacity has a definite role in the passage from NGT via IGT to type 2 diabetes in Japan (33).

Our group also showed relatively lower compensatory insulin secretion in Korean subjects, according to the elevation of fasting glucose levels. The maximum increments in insulin secretion according to the increasing fasting glucose levels reached only 150% of the basal levels regardless of the degree of obesity in our patients (Figure 1). However, the maximum compensatory increments in fasting insulin levels could reach almost 250% of the basal levels in Caucasian data (34). This difference might show a limited compensatory insulin secretory capacity in Korean subjects with type 2 diabetes mellitus. Furthermore, the acute insulin response to intravenous glucose infusion was completely abolished and no recovery of insulin response was observed, even in patients whose glucose tolerance had recovered from the diabetic range to the NGT or IGT range after strict blood glucose control (Figure 2). All of these results suggest that the loss of early-phase insulin secretion and limited capacity for compensatory insulin secretion could play a major pathogenic role in the development of abnormal glucose tolerance in our patients.

3.3. Decreased beta-cell mass in patients with type 2 diabetes

Although not much data are available, previous studies suggest that beta-cell mass is adaptively increased in nondiabetic obese humans (35, 36) and decreased in patients with type 2 diabetes mellitus. Butler *et al.* reported that the relative beta-cell volume in humans is decreased to almost 40% of that in normal subjects, even in IFG subjects. This observation implies that the deficit in the beta-cell volume is an early occurrence in the development of type 2 diabetes and is likely to be of primary importance rather than simply occurring secondary to hyperglycemia (8). We also demonstrated that beta-cell mass is decreased in diabetic patients. The mean relative volumes of beta-cells in diabetic patients were decreased by almost 50% compared with normal pancreases. However, when the relative volume of the pancreas was measured, alpha-cells accounted for 1.1 ± 1.0% of the whole pancreas in type 2 diabetic patients, almost twofold higher than the percentage of alpha-cells in normal subjects (7). The isl*et al*pha/beta ratio was found to be significantly elevated in these patients compared with normal subjects. Another interesting observation was the linear correlation between BMI and beta-cell mass in normal subjects ($r^2 = 0.64$; $P < 0.003$) and diabetic patients ($r^2 = 0.55$, $P < 0.05$). The relative beta-cell volumes in 16 diabetic patients whose BMIs were less than 25 kg/m^2 (64%) were lower than 50% of the mean value of the two control groups. The mean relative volume of the beta-cells in relatively obese type 2 diabetic patients (BMI: ≥ 25 kg/m^2; n = 4) reached 80% of the mean value of the control groups. However, no significant relationship was found between the relative volume of beta-cells and duration of diabetes ($r^2 = 0.118$, $P = 0.70$) and HbA1c.

To elucidate the underlying mechanism of the relative loss of beta-cell mass in type 2 diabetes, Butler *et al.* examined the frequency of new islet formation as well as beta-cell replication and apoptosis in islets. There was no

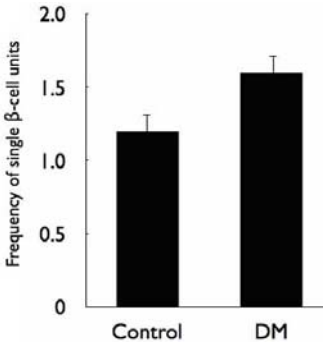

Figure 3. Frequency of single beta cell units found in normal (n=5) and diabetic patients (n=9). The frequency was 1.2 ± 0.52/44,000 μm^2 of islet area in normal subjects and 1.6 ± 0.52/44,000 μm^2 in diabetic patients (p=0.11).

difference in the percentage of exocrine duct cells positive for insulin in type 2 diabetic versus nondiabetic subjects, in either the lean or the obese groups. In our hands, the contribution rate of the beta-cell area of single beta-cell units, which were defined as islets composed of less than three cells and recognized as neogenetic loci (37, 38), to total beta-cell area showed a higher tendency in the type 2 diabetes mellitus group (7 ± 5% vs 10 ± 6%, control vs type 2 diabetes mellitus group, our unpublished data Figure 3) but there was no statistical difference. These results imply that new islet formation, the predominant input into the beta-cell mass in humans, appears to be intact in type 2 diabetes (39). Therefore, one might predict that the mechanism for the decreased beta-cell mass in type 2 diabetes is an increase in beta-cell apoptosis. Consistent with this suggestion, Butler *et al.* reported a threefold increased frequency of beta-cell apoptosis in obese cases of type 2 diabetes and a 10-fold increased frequency in lean cases of type 2 diabetes compared with nondiabetic control cases (8).

If the cause of the decreased beta-cell mass in type 2 diabetes is increased beta-cell apoptosis, what is the mechanism of increased alpha-cell mass and the alpha/beta ratio in the islets? As described above, in human pancreatic islets with type 2 diabetes, we can observe islets with selective beta-cell loss, some areas of which are replaced by fibrosis. From the previously reported findings, we can suggest a hypothesis for morphologic alteration of the islets in diabetic patients. Increased insulin resistance of various causes might stimulate beta-cell neogenesis from stem cells and self-replication of preexisting beta-cells, while acceleration in beta-cell apoptosis greater than the increase in the proliferation of beta-cells in diabetic patients results in a progressive decrease in the beta-cell mass over time. According to animal studies, the neogenesis of beta-cells in the adult usually recapitulates embryonic development (40). Therefore, we could expect that alpha-cell would also be newly formed during the neogenesis of beta-cells, as occurs in the embryonic development of the islets. After neogenesis of the alpha- and beta-cells, the beta-cells selectively disappear by accelerated apoptosis. Finally, we observed increased alpha-cell mass and selective loss of beta-cells with hyaline material deposition and fibrotic islet destruction, especially in large islets (Figure 4).

4. THE ROLE OF ACTIVATION OF PANCREAS STELLATE CELLS (PSC) ON FIBROTIC ISLET DESTRUCTION

4.1. Implications in the development and progression of type 2 diabetes mellitus

Regardless of underlying causes, morphologic changes resulting in severe insulin underproduction would be considered to follow destruction of normal islet architecture with fibrosis as well as apoptotic beta-cell loss. Islet fibrosis with reduced beta-cell mass is frequently observed in animal models of type 2 diabetes, such as in Otsuka Long Evans Tokushima Fatty (OLETF) and Zucker Diabetic Fatty (ZDF) rats, and db/db mice. We also observed some destructive changes in the pancreatic islets of type 2 diabetic patients (7). Many researchers have highlighted the role of amylin, which is cosecreted with insulin from the beta-cells and might have harmful effects on the beta-cells (41-43); however, the role of fibrotic islet destruction has been ignored to date. We propose that fibrotic islet destruction might be an important cause of limited beta-cells proliferation in diabetic patients. As is well known, tissue fibrosis is one of the limiting factors for cell proliferation and regeneration in many tissues (44, 45).

Pancreatic stellate cells (PSCs) and angiotensin receptors are present in the islets (46) and long-term treatment with the angiotensin-converting enzyme (ACE) inhibitor (ACEi) ramipril in an animal model of type 2 diabetes showed much improved glucose tolerance test and reduced islet fibrosis (47, 48). Significant activation of PSCs in the islets of diabetic patients has also been noted. All of these results suggest that islet fibrosis and beta-cell loss advance over time, and that protection from islet fibrosis might be a strategy to halt the progression of type 2 diabetes.

4.2. Islet fibrosis and PSC

In our previous report, the ACEi ramipril significantly attenuated islet fibrosis in OLETF rats, an animal model of type 2 diabetes mellitus (47). Interestingly, the proliferation of alpha smooth muscle actin (alpha-SMA)-positive PSCs, fibrosis of the pancreatic islets, and extracellular matrix production in the islets increased significantly in OLETF rats, and these changes were attenuated by ramipril treatment (Figure 5). We found prominent islet fibrosis with destroyed islet architecture, which was accompanied by alpha-SMA-positive cells in an advanced type 2 diabetes mellitus animal model without evidence of pancreatitis. OLETF rats with diabetic progression display severe islet destruction because of fibrosis, which is accompanied by increased pancreatic expression of alpha-SMA, a specific marker of PSCs, especially surrounding the destroyed islets (46, 49). These data suggest that PSCs have a role in pancreatic islet fibrosis in models of type 2 diabetes mellitus, although this needs to be clarified.

Since their discovery in 1998, PSCs have been

Loss of beta-cells with fibrotic islet destruction

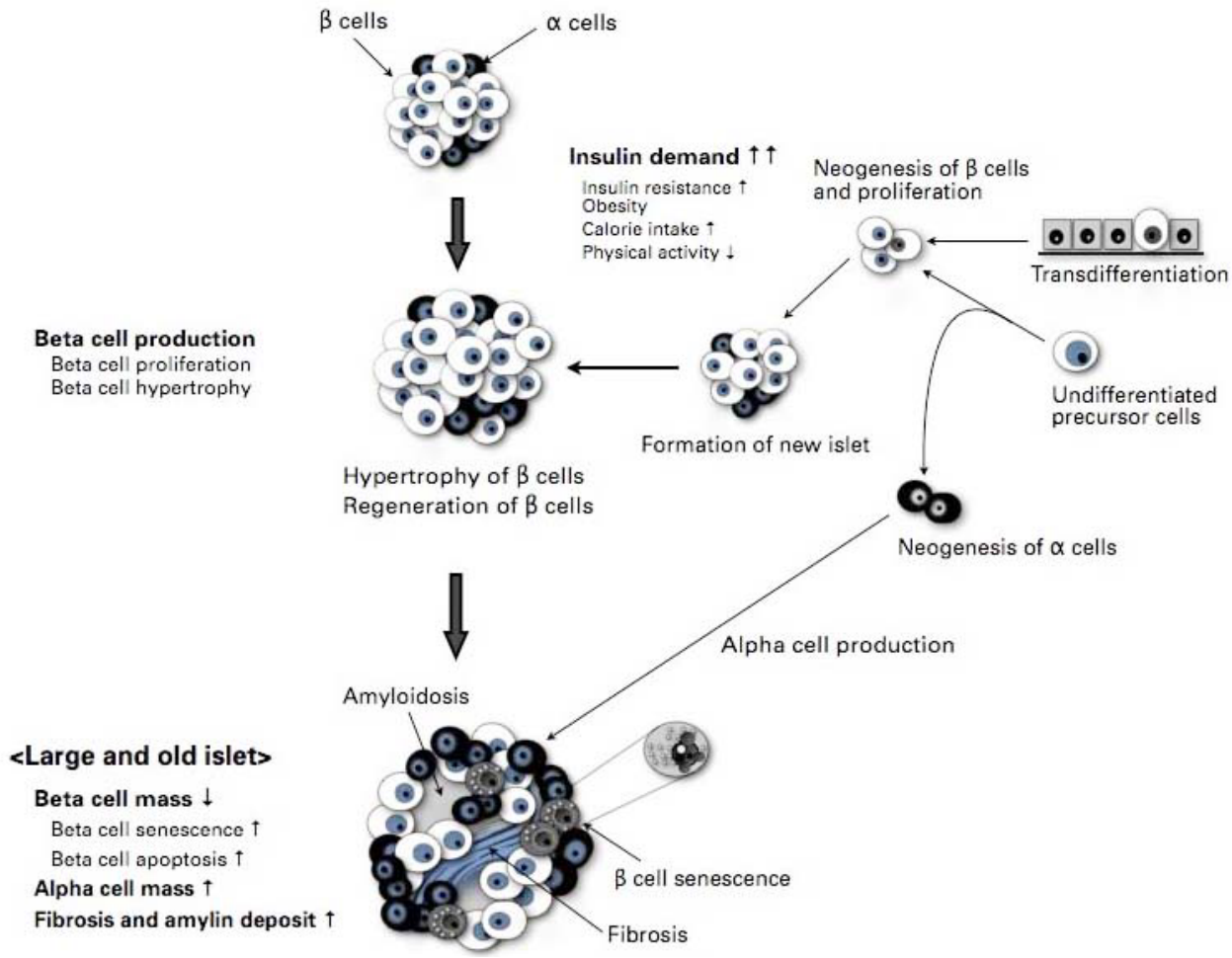

Figure 4. Hypothesis for the morphologic alterations of islets in diabetic patients. Increase insulin resistance with various causes might stimulate beta-cell neogenesis from stem cells and regeneration of preexisting adult beta cell, while more accelerated beta-cell apoptosis than increasing the neogenesis and self-replication of beta-cell resulted progressive decreasing the beta-cell mass over time. According to the animal studies, the neogenesis of beta-cell in the adult usually recapitulation of the developmental process of the embryonic day. So we could expect that alpha-cell also newly formed during neogenesis of beta-cells. After neogenesis of the alpha- and beta-cells, beta-cells were selectively disappeared by accelerated apoptosis. Finally we observed increased the alpha-cell mass and selective loss of beta-cell with hyaline material deposition and fibrotic islet destruction especially in the large islets.

identified as the major source of the extracellular matrix (ECM) proteins found in chronic pancreatitis or pancreatic fibrosis in both experimental models and humans (50). In the quiescent state, PSCs contain vitamin A-storage droplets in their cytoplasm. When activated by cytokines or oxidative stress, PSCs transform into myofibroblast cells and stain positive for alpha-SMA. PSCs markedly increase ECM protein synthesis when activated by cytokines or growth factors. Multiple recent studies report that activated PSCs might play a role in pancreatic fibrogenesis, including in chronic pancreatitis and alcoholic pancreatic fibrosis. We have also reported that ACEis attenuate islet destruction by fibrosis and have suppressive effects on ECM protein expression: these effects are accompanied by the suppression of alpha-SMA (47). These findings imply that islet fibrosis and PSC proliferation are related to the renin–angiotensin system (RAS).

Pancreatic stellate cells and local rennin-angiotensin system, with angiotensin receptors, are present in pancreatic islets (46) and long-term treatment with the ACEi ramipril in an animal model of type 2 diabetes led to much improved glucose tolerance and reduced islet fibrosis (47). Significant activation of PSCs in the islets of diabetic patients has also been noted. All of these results suggest that islet fibrosis and beta-cell loss advance over time, and that protection against islet fibrosis might be a strategy to halt the progression of type 2 diabetes.

4.3. Effects of glucose on the expression of Renin-angiotensin system (RAS) in the pancreas and PSCs

Beyond its hemodynamic effects, angiotensin II (Ang II) plays an important role in tissue inflammation. Locally produced Ang II promotes recruitment of inflammatory cells, induces the expression and secretion of

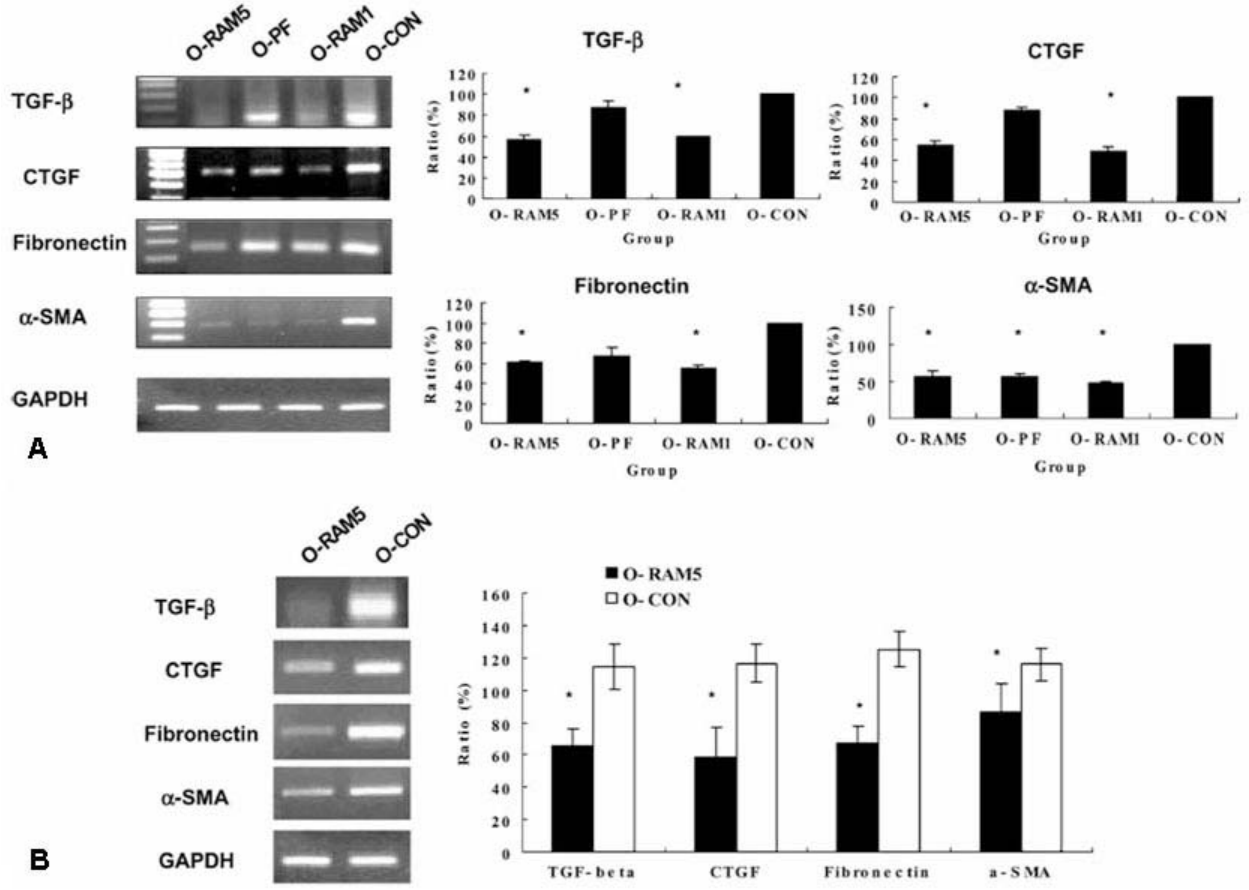

Figure 5. Reverse transcription polymerase chain reaction analysis after ramipril treatment in OLETF rats for the expression of TGF-beta, connective tissue growth factor (CTGF), and fibronectin mRNAs at 24 weeks. O-RAM5, lane 1; O-PF (paired-feeding group), lane 2; O-RAM1, lane 3; and O-CON, lane 4 (A). At 32 weeks, ramipril treatment showed a clear reduction of expression of mRNAs compared with the control group in the pancreas of OLETF rats. O-RAM5, lane 1; O-CON, lane 4 (B). *p < 0:05 vs. O-CON group.

ECM proteins, and inhibits collagen degradation. The RAS is believed to be involved in tissue remodeling and fibrogenesis in the kidney, heart, liver, and especially, the pancreas. The pathogenic mechanism of diabetic nephropathy could be partly explained by RAS activation leading to mesangial cell proliferation, eventual progressive renal hypertrophy, and renal fibrosis. In addition to diabetic nephropathy, vascular changes with smooth muscle cell proliferation are prominent in diabetic conditions. In this pathogenesis, the role of Ang II is considered a key factor, acting as an inflammatory mediator.

What is the role of Ang II in pancreatic islets of diabetes mellitus? Previous reports showed that Ang II infusion attenuated insulin secretion or decreased insulin sensitivity. A local RAS with its major components, such as rennin-angiotensinogen, and Ang II and its receptors, has been identified in pancreatic islets. Moreover, as in the case of renal fibrosis, we observed that blocking of RAS in OLETF rats attenuated pancreatic islet fibrosis. These findings suggest that the intra-islet RAS could influence the pathogenesis of islet fibrosis.

The mechanisms underlying the Ang II increase in response to glucose have not been clarified. In the proximal tubule cells of the kidney, a glucose response element has been identified in the angiotensinogen gene promoter, and high glucose stimulates angiotensinogen synthesis in a concentration-dependent manner (51-53). In mesangial cells, high glucose concentrations increase Ang II generation due to an increase in intracellular renin activity, mediated by the time-dependent stimulation of (pro)renin gene transcription, the reduction in prorenin enzyme secretion, or an increased rate of conversion of prorenin to active renin (54). However, there is currently little evidence that hyperglycemia can lead to islet fibrosis by activation of RAS or Ang II production.

As previously described, RAS components including AT receptors, renin, angiotensignoen and Ang II are also localized in PSCs. We have shown that PSCs are activated by high glucose concentrations and that PSC

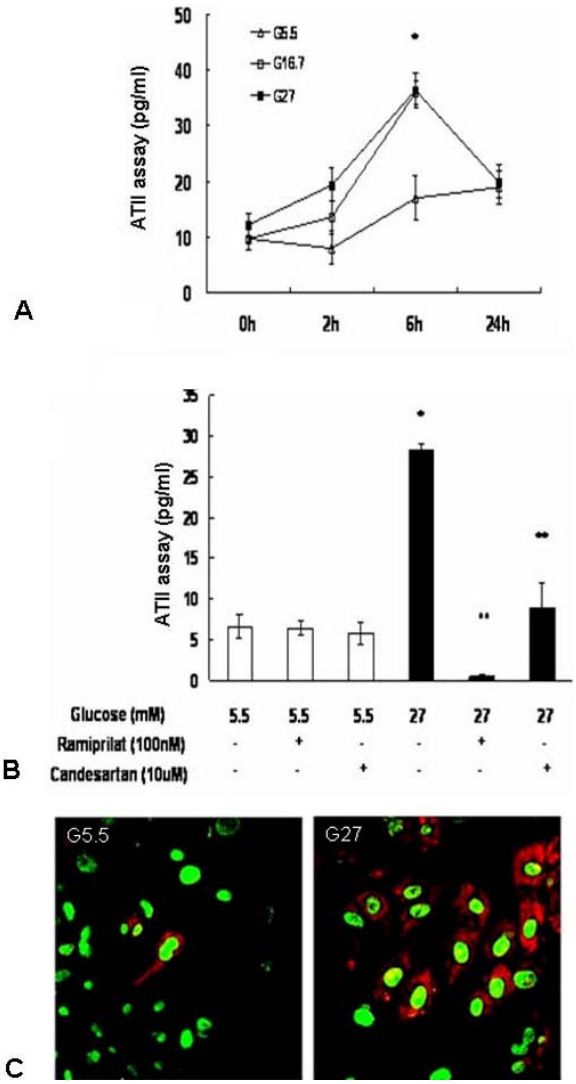

Figure 6. Effect of glucose on Ang II concentration in PSCs. (A) Ang II concentration increased significantly under high-glucose conditions. (B) PSCs treated with ramipril or candesartan showed significantly attenuated increases in Ang II concentration under high-glucose conditions. (C) The number of immunostained Ang II-positive cells increased significantly in the high glucose concentration. Reprinted with permission from J Cell Biochem.

proliferation following high-glucose stimulation is accompanied by Ang II production. In addition, high-glucose concentration- induced Ang II production was virtually abolished by preincubation of the PSCs with an ACEi and an angiotensin receptor antagonist (46). Therefore, we suggest that PSCs would be one of the candidates for leading to pancreatic islet fibrosis in type 2 diabetic animal model. As in case of mesangial cells in diabetic renal complication, hyperglycemia might induce PSCs activation by Ang II overproduction. Decrease of Ang II production by ramipril treatment of PSCs was higher compared to candesartan treatment (Figure 6). Further studies are required to clarify the exact mechanism responsible for the effect of high glucose concentration on Ang II production in PSCs.

5. SPECIFIC FIBROTIC ISLET DESTRUCTION BY PSC IN DIABETIC PATIENTS

5.1. Might hyperglycemic and hyperinsulinemic environment of islet be the cause?

In contrast to acute or chronic pancreatitis, in which fibrosis mainly involves the exocrine pancreatic tissue, pancreatic islet fibrosis in people with type 2 diabetes mellitus is confined mainly to the endocrine pancreatic islet tissue, even though the entire pancreas is exposed to hyperglycemia and PSCs also exist in exocrine pancreas. One possibility for islet specific fibrosis in patients with diabetes is that PSCs in the islets are exposed to both hyperglycemia and hyperinsulinemia. Insulin and insulin-like growth factor 1 are well-known mitogens for fibroblasts and smooth muscle cells, and PSCs in the islets might be predisposed to activation and proliferation induced by hyperglycemia, hyperinsulinemia, or both.

Insulin is a potent cell growth factor and is secreted continuously at a relatively high concentration into the capillaries within the islets, although relative insulin deficiency in the whole body occurs in type 2 diabetes mellitus. We hypothesized that local hyperinsulinemia in the islets might predispose toward PSC activation and proliferation in a hyperglycemic environment. In a recent study, we demonstrated that glucose is more potent than insulin and enhances PSC proliferation gradually in a dose- and time-dependent manner. Although not as effective as glucose, insulin also significantly influences PSC proliferation within a limited concentration range. Combined treatment of PSCs with glucose and insulin produced a peak in proliferation that was nearly six times the basal level, confirming the additive effect of glucose and insulin (55) (Figure 7).

The signaling pathways activating stellate cells are not fully understood, although several studies have shown that the ERK (56) and the p38 MAPK (mitogen-activated protein kinase) pathways (57) were involved. In our study, glucose and insulin induced ERK 1/2 phosphorylation in a dose-dependent manner. Moreover, connective tissue growth factor, an important downstream mediator of TGF-beta activity (58), was significantly upregulated by high glucose and insulin concentrations and was nearly completely suppressed by the MAPK inhibitor, U0126.

5.2. Islet fibrotic destruction with PSC activation in human pancreatic tissue with type 2 diabetes

The clinical implications of the activation of PSCs in the pathogenesis of type 2 diabetes have been investigated infrequently. We examined pancreatic sections from patients with type 2 diabetes and found increased and prominent intra-isl*et al*pha-SMA immunostaining (Figure 8B), especially in distorted islets, compared with staining in

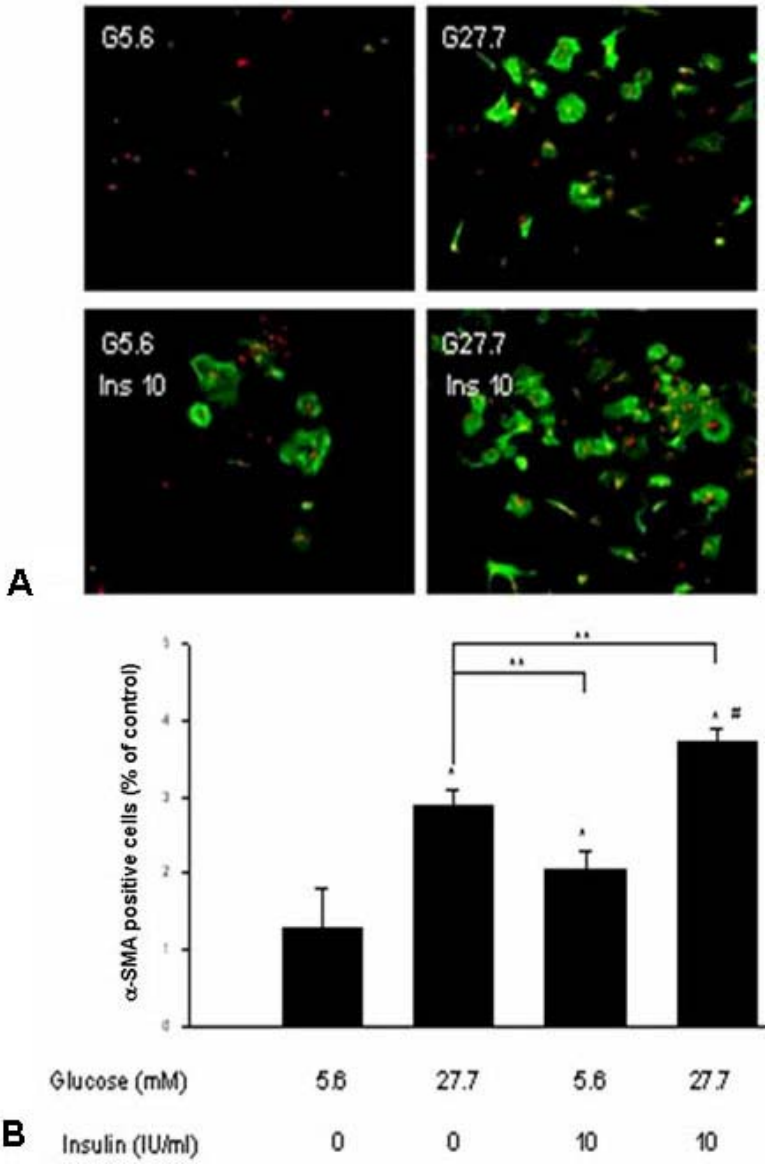

Figure 7. Effect of high glucose and insulin concentrations on the activation of quiescent PSCs assessed by immunostaining (A) and western blotting (B) with anti-alpha-SMA antibody. Combined stimulation by glucose (27.7 mM) and insulin (10 IU/ml) markedly increased the expression of alpha-SMA, a specific marker of PSCs. * $p < 0.05$ vs. 5.6 mM glucose; ** $p < 0.05$ vs. 27.7 mM glucose; # $p < 0.05$ 5.6 mM glucose + 10 IU/ml insulin vs. 27.7 mM glucose + 10 IU/ml insulin (unpublished data).

a sample from a healthy person (Figure 8A). The clinical significance of this finding should be further evaluated.

In summary, it appears that hyperglycemia and hyperinsulinemia are the two crucial mitogenic factors that induce the proliferation of PSCs, and the presence of these two factors at the same time probably amplifies PSC activation and proliferation. Ang II may also aggravate the process of islet fibrotic changes, eventually leading to islet fibrosis. Therefore, rigorous control of the blood glucose concentration and improvement of the insulin resistance associated with diabetes may suppress fibrosis of the pancreas and pancreatic beta-cell loss.

6. CONCLUSTION

Recent morphologic analyses of human pancreases strongly suggest that a decreased beta-cell mass is observed from the early stages of diabetes. In this article, we propose that fibrotic islet destruction might be one of the important pathogenic mechanisms of the limited capacity of beta-cell proliferation in diabetic patients. We have found that PSCs are involved in the progression of islet fibrosis in an animal model of type 2 diabetes and, possibly, in human with type 2 diabetes. Both of high concentrations of glucose and insulin in islets contribute to PSC activation and proliferation in diabetis patients,

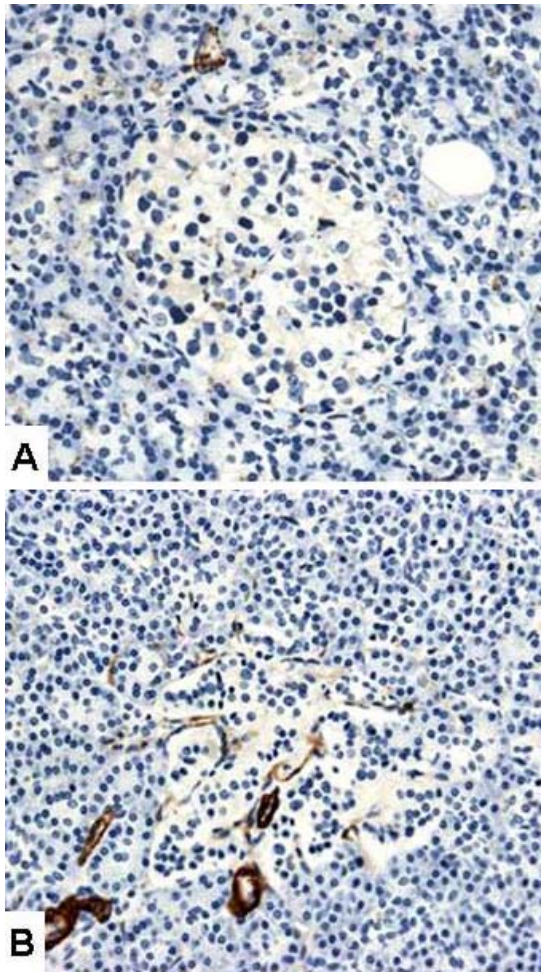

Figure 8. Immunostaining of alpha-SMA in a pancreatic section from a healthy human (A) and a patient with type 2 diabetes mellitus (B). Compared with the sample from the healthy person, the pancreatic islets of the diabetic patient show increased expression of alpha-SMA (brown colour) especially in the pancreatic islets.

although the exact mechanisms remain to be confirmed. Both *in vitro* and *in vivo* studies indicate that ACEis attenuate islet destruction caused by fibrosis and that these have some beneficial effects on glucose tolerance by suppressing PSC activation and proliferation. We suggest that PSCs might play an important role in the pathogenesis of fibrotic islet destruction observed in type 2 diabetes.

7. ACKNOWLEDGEMENT

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