

Pancreatic stellate cells in the islets as a novel target to preserve the pancreatic β -cell mass and function

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ABSTRACT

There are numerous lines of clinical evidence that inhibition of the renin–angiotensin system (RAS) can prevent and delay the development of diabetes. Also, the role of RAS in the pathogenesis of diabetes, including insulin resistance and β -cell dysfunction, has been extensively investigated. Nevertheless, this role had not yet been fully shown. A variety of possible protective mechanisms for RAS blockers in the regulation of glucose homeostasis have been suggested. However, the direct effect on pancreatic islet fibrosis has only recently been spotlighted. Various degrees of islet fibrosis are often observed in the islets of patients with type 2 diabetes mellitus, which can be associated with a decrease in β -cell mass and function in these patients. Pancreatic stellate cells are thought to be deeply involved in this islet fibrosis. In this process, the activation of RAS in islets is shown to transform quiescent pancreatic stellate cells into the activated form, stimulates their proliferation and consequently leads to islet fibrotic destruction. In this article, we introduce existing clinical and experimental evidence for diabetes prevention through inhibition of RAS, and review the responsible local RAS signaling pathways in pancreatic stellate cells. Finally, we propose possible targets for the prevention of islet fibrosis.

INTRODUCTION

Type 2 diabetes mellitus is a heterogeneous metabolic disease entity that shares its phenotype of hyperglycemia, and fundamental pathophysiology of insulin resistance and impaired β -cell function. Over the past decades, insulin resistance was thought to be the main contributor to type 2 diabetes mellitus, and decreased insulin secretion was considered as a part of the late manifestation in the course of the disease¹. However, there has been abundant evidence that individuals with type 2 diabetes mellitus already have both a significant decrease in β -cell function^{2,3} and a loss of 30–40% β -cell mass, even at the time of diagnosis^{4,5}. It is now well recognized that decompensation of β -cells to increase insulin secretion compared with insulin resistance leads to overt hyperglycemia, indicating that β -cell dysfunction is critical to the development of type 2 diabetes mellitus^{6,7}. Furthermore, as β -cell dysfunction accelerates over time, it should be focused on as a significant determinant of the progression rate of type 2 diabetes mellitus⁸.

Recent evidence suggests that there are ethnic differences in the pathophysiological characteristics of type 2 diabetes mellitus. Asians typically have lower levels of obesity, but a more significantly reduced incremental insulin release in response to insulin resistance compared with Caucasians^{9,10}. Combining these backgrounds, we should emphasize the preservation of β -cell mass and function to preventing the development and progression of type 2 diabetes mellitus, especially in Asians.

The causes of the loss of β -cell mass and function are numerous. Glucolipototoxicity, chronic inflammation, endoplasmic reticulum stress, oxidative stress, amyloid deposition and epigenetic modifications have all been linked to the initiation and progression of β -cell impairment and loss^{11–13}. Also, there is evidence that islet disorganization through progressive fibrosis might also be an important mechanism of β -cell dysfunction and loss^{14,15}. Fibrosis is the well-known pathogenic process that results in progressive loss of the structure and function of the affected organs¹⁶. Progressive fibrosis and the subsequent loss of functional tissue (replaced by extracellular matrix [ECM]-rich connective tissue containing amylin) is also well demonstrated in the pathogenesis of chronic pancreatitis and pancreatic ductal adenocarcinoma¹⁷. In this process of pancreatic

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fibrogenesis, pancreatic stellate cells (PSCs) have been identified as a major source of ECM proteins¹⁸.

Besides exocrine pancreatic fibrosis, pancreatic islet fibrosis has also been identified in both animal models and individuals with diabetes^{5,19}. Interestingly, pancreatic fibrosis in diabetes is mostly restricted to pancreatic islets compared with the fibrosis of exocrine pancreatic tissue in chronic pancreatitis, suggesting that islet fibrosis in diabetes is linked to activating pathways that are different from those of the exocrine pancreatic disease^{20,21}. In our basic research, we found that activation of PSCs played a crucial role in the process of islet fibrosis through the angiotensin II (Ang II) signaling pathway^{20,22}. However, the exact mechanism of fibrogenesis in pancreatic islets and the involvement of PSCs in this process have been much less studied and highlighted, despite their importance.

In the present review, we introduce the existing clinical and experimental evidence of diabetes prevention through inhibition of the renin–angiotensin system (RAS), and discuss the responsible RAS signaling pathways in PSCs. Finally, we introduce possible targets for the prevention of islet fibrosis.

CLINICAL STUDIES SHOWING A PREVENTIVE EFFECT OF RAS INHIBITION ON DIABETES

Diabetes represents a global epidemic whose incidence is steadily increasing, and therefore, there are ongoing efforts worldwide aimed at preventing or delaying the onset of type 2 diabetes mellitus. The two main strategies are lifestyle modifications and pharmacological interventions. Most of the pharmacological evidence comes from the use of antidiabetic drugs, such as metformin, thiazolidinediones, α -glucosidase inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonist²³, or antiobesity drugs, such as orlistat²⁴. Another potential pharmacological strategy is the use of RAS blockers, which are antihypertensive drugs. Although other antihypertensive drugs, such as diuretics and β -adrenergic blockers, have adverse effects on insulin resistance and glucose tolerance²⁵, RAS blockers have shown favorable results by preventing the onset of diabetes in numerous clinical trials on individuals with hypertension or other cardiovascular risk factors (Table 1).

The Captopril Prevention Project was a randomized and open-label study that compared captopril, an angiotensin-converting enzyme inhibitor (ACEi), with conventional antihypertensive agents (diuretics and β -blockers)²⁶. The incidence of diabetes was lower in the captopril group than in the conventional treatment group (relative risk 0.86, 95% confidence interval [CI] 0.74–0.99; $P = 0.039$). Similar results have been observed in other trials. The Heart Outcomes Prevention Evaluation study was a randomized, placebo-controlled trial²⁷ in which ramipril, an ACEi, showed a risk reduction for diabetes (relative risk 0.66, 95% CI 0.51–0.85; $P < 0.001$). The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial^{28,29} and the Losartan Intervention For End Point Reduction in Hypertension trial³⁰ were randomized, double-blind studies that compared the ACEi, lisinopril, with either the

diuretic, chlorthalidone, or calcium channel blocker, amlodipine, and the angiotensin receptor blockers (ARB), losartan, with the β -blocker, atenolol, respectively. The risk reduction for diabetes in both trials was ~30% (lisinopril vs chlorthalidone, $P < 0.001$), 17% (lisinopril vs amlodipine, $P < 0.01$) and 25% (losartan vs atenolol, $P < 0.001$), respectively. The Study on Cognition and Prognosis in the Elderly (SCOPE)³¹, which compared the ARB candesartan with a placebo, showed a tendency toward risk reduction for diabetes in elderly hypertensive patients (25%, $P = 0.09$). More recently, the Valsartan Antihypertensive Long-Term Use Evaluation trial³² was a randomized, double-blinded study that compared the ARB, valsartan, with amlodipine. The Valsartan Antihypertensive Long-Term Use Evaluation trial also produced positive results for the onset of diabetes (odds ratio 0.77, 95% CI 0.69–0.87; $P < 0.0001$). Taken together, these clinical studies suggest that inhibition of RAS with either an ACEi or an ARB might protect high-risk individuals (with hypertension or other cardiovascular risk factors) from the development of type 2 diabetes mellitus. Among these studies, the Heart Outcomes Prevention Evaluation and Valsartan Antihypertensive Long-Term Use Evaluation trials provided the strongest evidence, because these trials were carried out using a placebo or calcium channel blocker, which is considered metabolically neutral.

Because all the positive data obtained in these clinical studies came from comparisons with other antihypertensive drugs or resulted from the use of a secondary end-point or post-hoc analysis, more direct clinical proof of the preventive effect of RAS blockade on the onset of diabetes was required. This situation resulted in the initiation of two large, double-blind, placebo-controlled, randomized trials whose primary outcome was the development of type 2 diabetes mellitus: the Diabetes Reduction Approaches With Ramipril and Rosiglitazone Medications (DREAM) trial using the ACEi, ramipril, as well as the Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) trial using the ARB, valsartan^{33,34}. Frustratingly, the DREAM trial failed to show any difference in the incidence of new-onset diabetes (hazard ratio for the ramipril group 0.91, 95% CI 0.81–1.03; $P = 0.15$)³³. In contrast, the NAVIGATOR trial showed a protective effect of ARB as compared with the placebo group (hazard ratio for the valsartan group 0.86, 95% CI 0.80–0.92; $P < 0.001$)³⁴.

Except for the administration of an ACEi or an ARB, these two large trials differed in several respects. First, unlike the other trials, DREAM included only patients who had impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), but no known cardiovascular disease (CVD). In contrast, the NAVIGATOR trial, like the previous trials, included patients with IFG and also with established CVD or cardiovascular risk factors. Nearly 99% of the participants in the NAVIGATOR trial had at least one cardiovascular risk factor, including a history of CVD (24.3%) and hypertension (79.6%). Thus, the participants in the NAVIGATOR trial were expected to have a higher RAS activity, a risk factor for the development of

Table 1 | Summary of clinical studies showing the effect of RAS inhibition on new-onset type 2 diabetes mellitus

Trial	Characteristics of participants	Comparison groups		No. participants in analysis [†]	Duration of follow up (years)	Relative risk (95% confidence interval)	Reference
		Treatment group	Control group				
CAPPP	Aged 25–66 years with diastolic hypertension	Captopril	Diuretics, β -blockers	5,183 vs 5,230	Mean 6.1	0.86 (0.74–0.99)	26
HOPE	Aged ≥ 55 years with coronary artery disease, stroke, peripheral vascular disease	Ramipril	Placebo	2,837 vs 2,883	Median 4.5	0.66 (0.51–0.85)	27
ALLHAT	Aged ≥ 55 years with hypertension and at least one other coronary heart disease risk factor	Lisinopril	Chlorthalidone Amlodipine	2,567 vs 4,543 2,567 vs 2,692	Median 4.9	0.70 (0.63–0.77) 0.83 (0.74–0.93)	28,29
LIFE	Aged 55–80 years with hypertension and left ventricular hypertrophy	Losartan	Atenolol	4,019 vs 3,979	Mean 4.8	0.75 (0.63–0.88)	30
SCOPE	Aged 70–89 years with hypertension	Candesartan	Placebo	2,167 vs 2,175	Mean 3.7	0.75 (NA), $P = 0.09$	31
CHARM	Aged >18 years with heart failure NYHA grade II–IV	Candesartan	Placebo	2,715 vs 2,721	Median 3.1	0.78 (0.64–0.96)	88
PEACE	Aged ≥ 50 years with stable coronary artery disease and left ventricular ejection fraction >40%	Trandolapril	Placebo	3,432 vs 3,472	Median 4.8	0.83 (0.72–0.96)	89
VALUE	Aged ≥ 50 years with hypertension and high risk of cardiovascular events	Valsartan	Amlodipine	5,032 vs 4,963	Mean 4.2	0.77 (0.69–0.87)	32
DREAM [‡]	Aged ≥ 30 years without cardiovascular disease but with impaired fasting glucose or impaired glucose tolerance	Ramipril	Placebo	2,623 vs 2,646	Median 3.0	0.91 (0.81–1.03)	33
ONTARGET	Aged ≥ 55 years with coronary, peripheral artery or cerebrovascular disease	Ramipril + Telmisartan	Ramipril	5,280 vs 5,427	Median 4.7	0.91 (0.78–1.06)	90
TRANSCEND	Aged ≥ 55 years with coronary, peripheral artery or cerebrovascular disease	Telmisartan	Placebo	1,895 vs 1,913	Median 4.7	0.85 (0.71–1.02)	90
NAVIGATOR [‡]	Aged ≥ 50 years with impaired glucose tolerance and established cardiovascular disease or cardiovascular risk factors	Valsartan	Placebo	4,631 vs 4,675	Median 5.0	0.86 (0.80–0.92)	34
CASE-J Ex	20–Aged 85 years, Japanese with hypertension and at least one risk factor for cardiovascular events	Candesartan	Amlodipine	636 vs 620	Mean 4.5	0.71 (0.51–1.00)	91
ANBP2	Aged 65–84 years with hypertension, but having no recent cardiovascular morbidity (within 6 months)	Enalapril	Hydrochlorothiazide	2,815 vs 2,827	Median 6.9	0.70 (0.56–0.86)	92,93

[†]Number of participants included in the analysis of secondary outcomes was estimated as the number of total participants – the number of participants with type 2 diabetes mellitus at baseline, if there was no information in the original article. [‡]Only Diabetes Reduction Approaches With Ramipril and Rosiglitazone Medications (DREAM) and Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research (NAVIGATOR) were the double-blind, placebo-controlled, randomized trials whose primary outcome was the development of type 2 diabetes mellitus. CAPPP, Captopril Prevention Project; HOPE, Heart Outcomes Prevention Evaluation; LIFE, Losartan Intervention For End Point Reduction in Hypertension; NA, not available; NYHA, New York Heart Association; SCOPE, Study on Cognition and Prognosis in the Elderly; VALUE, Valsartan Antihypertensive Long-Term Use Evaluation.

diabetes, as compared with the patients in the DREAM trial who were not at the same risk. Second, the study period differed between the two trials. The median follow-up period was 3.0 and 5.0 years in DREAM and NAVIGATOR, respectively. Perhaps due to the relatively short period of the study, the DREAM trial could only show the potential for efficacy. In this regard, although the DREAM trial failed to yield positive results in terms of the effect on the incidence of new diabetes, 42.5% of patients in the ramipril group converted from IFG or IGT to normoglycemia, and this improvement was higher than the 38.2% conversion seen in the placebo group ($P = 0.001$).

In the meta-analyses of clinical studies, both ACEis and ARBs showed a consistent significant risk reduction in new-onset diabetes in the various subgroups of combining CVDs, except for the individuals with IFG or IGT, due to the negative results of the DREAM trial^{35,36}. Interestingly, the reduced risk of new-onset diabetes was irrespective of achieved blood pressure levels in both ACEis and ARBs.

HYPOTHETICAL MECHANISMS EXPLAINING THE PREVENTIVE EFFECTS OF RAS INHIBITION ON DIABETES

In addition to clinical evidence, there is abundant *in vitro* and *in vivo* evidence that has clearly shown the adverse effects of RAS activation on insulin secretion and sensitivity, and also the possibility of reversal of these effects through RAS blockade. In a study with *db/db* mice, the upregulated pancreatic islet Ang II receptor type 1 (AT1R) was accompanied with deleterious effects on insulin secretion and (pro)insulin biosynthesis³⁷. In rodents, exposure of isolated islets to Ang II induced a dose-dependent inhibition of glucose-stimulated insulin secretion and (pro)insulin biosynthesis. This inhibitory action was completely prevented by pretreatment with losartan³⁸. In humans, a 3-month treatment with candesartan was shown to increase first-phase insulin secretion during an oral glucose tolerance test³⁹. Additionally, in a study on a hyperinsulinemic-euglycemic and hyperglycemic clamp, 26 weeks of treatment with valsartan increased both glucose-stimulated insulin secretion and insulin sensitivity in normotensive individuals with IGT⁴⁰.

Although abundant evidence has been accumulated, the precise molecular mechanism by which RAS inhibition affects the pathogenesis of diabetes has not yet been elucidated. A variety of potential mechanisms have, therefore, been proposed to explain the preventive and delaying effects of RAS inhibition on diabetes. These include physiological changes that might improve insulin sensitivity and secretion, and also direct effects on changes in islet morphology.

First, RAS inhibition-mediated vasodilation might facilitate insulin secretion and insulin action by improving muscular and pancreatic blood flow. Both ACEis and ARBs have been reported to enhance blood flow in peripheral tissues, such as skeletal muscle, and this change might then improve insulin sensitivity and facilitate glucose disposal^{41,42}. Second, RAS inhibition could directly affect insulin signaling and improve insulin

sensitivity in skeletal muscle. Alterations in post-receptor insulin signaling in type 2 diabetes mellitus have been shown, including anomalies in phosphatidylinositol-3 kinase–protein kinase B signaling. There is evidence that Ang II aggravates these abnormalities. Therefore, RAS inhibition might have a direct effect on insulin signaling and regulation of glucose transporters⁴³. Third, RAS inhibition could also improve cellular insulin signaling and insulin sensitivity by reducing the levels of free fatty acids, and by inhibiting Ang II-mediated oxidative stress. Given that Ang II activates nicotinamide adenine dinucleotide phosphate oxidase, a major source of reactive oxygen species (ROS), increased RAS activity in β -cells may aggravate oxidative stress-induced β -cell dysfunction and apoptosis. ARBs have been reported to attenuate fatty acid-induced oxidative stress and nicotinamide adenine dinucleotide phosphate oxidase activity in pancreatic β -cells^{44–46}. In addition, a subset of AT1R blockers have been shown to induce peroxisome proliferator-activated receptor-gamma (PPAR- γ) activity by interaction with the PPAR- γ ligand-binding domain, thus they can improve insulin sensitivity⁴⁷. For example, telmisartan, one of the selective AT1R blockers, shares structural similarity with pioglitazone, and is known to act as a partial PPAR- γ agonist^{48,49}. However, it is unclear whether this effect is a class effect of ARBs. Finally, it has been shown that Ang II promotes lipid deposition in adipose tissue by inhibiting lipolysis and promoting lipogenesis, and also increases secretion of adipose tissue-derived proinflammatory cytokines. Thus, RAS inhibition might selectively alleviate insulin resistance in adipose tissue⁵⁰.

As discussed, many studies explaining the participation of RAS in the modulation of glucose homeostasis have focused on insulin sensitivity, but recently, the possibility of RAS having a direct influence on pancreatic islet fibrosis has gained attention. There has been enough evidence of the presence of Ang II receptors on the surface of pancreatic islet β -cells in rats and humans⁵¹. Thus, we initially hypothesized that RAS blockers might have a direct influence on pancreatic islets, eventually conserving the β -cell mass and function. Although the restoring of insulin sensitivity is essential, this mechanism alone could not fully explain the findings of increased insulin secretion after the administration of RAS blockers.

EFFECT OF RAS INHIBITION ON ISLET FIBROSIS AND PRESERVATION OF β -CELL MASS AND FUNCTION

The RAS is well-known for its classic function in the systemic regulation of blood pressure, fluid retention and electrolyte balance through action on vascular smooth muscle cells and aldosterone secretion. In the RAS signaling cascade, Ang II is the main effector peptide that acts mainly through AT1R. Also, Ang II acts as a growth factor that promotes cell growth and tissue inflammation, by which it plays a pivotal role in various disease-associated target organ fibrosis and dysfunction^{52,53}. For example, it has been well demonstrated that inhibition of RAS with ACEis or ARBs can delay the progression of diabetic nephropathy and prevent renal fibrosis⁵⁴. Also, inhibition of

RAS has been consistently shown to reduce endothelial dysfunction and atherosclerosis by suppression of inflammation, fibrosis and oxidative stress⁵⁵. Like other organ damage processes, we observed some similar destructive fibrotic changes in the pancreatic islets of both animal models of type 2 diabetes mellitus and patients with type 2 diabetes mellitus^{5,21} and thought the local RAS might be the main contributor to this phenomenon. There is abundant evidence that overactivation of local RAS also exists in the pancreas like other organs and tissues, including the brain, heart, kidney, adrenal glands, adipose tissue and skeletal muscle^{51,56}.

Otsuka Long Evans Tokushima fatty (OLETF) rats are an established rat model of type 2 diabetes mellitus in which extensive connective tissue proliferation in the pancreas is associated with pancreatic islet cell atrophy⁵⁷. We initially found that long-term ramipril treatment (24 weeks) prevented islet destruction caused by fibrosis in diabetic OLETF rats. These effects were accompanied with a decreased expression of transforming growth factor-beta (TGF- β) and its downstream signaling molecules, such as connective tissue growth factor (CTGF), fibronectin and alpha-smooth muscle actin (α -SMA)²⁰. However, although long-term ramipril treatment was shown to alleviate pancreatic islet fibrosis, the involvement of the islet RAS in islet fibrosis was not evident in OLETF rats. Upregulation of intra-islet AT1R and ACE was then shown by immunohistochemistry and real-time polymerase chain reaction analysis of the whole pancreas from another type 2 diabetes mellitus rat model, Zucker diabetic fatty rats⁵⁸. The increased intra-islet expression of components of RAS correlated with increased intra-islet fibrosis, apoptosis and oxidative stress. In addition, the RAS blockade significantly reduced islet fibrogenesis and improved islet architecture. These effects were associated with the attenuation of TGF- β and profibrotic pathways. Finally, improvements in structural parameters were associated with a significant improvement of first-phase insulin secretion.

In conclusion, RAS blockers appear to have a direct effect on pancreatic islet cells, such as the prevention of islet fibrosis and the maintenance of cellular architecture, which ultimately conserves the β -cell mass and function, thereby exerting a beneficial action on glucose tolerance. Thus, we highlight the reduction in islet fibrosis as an important and promising mechanism behind the long-term protective effects of RAS blockers on the development and progression of diabetes.

ROLE OF PSCS IN ISLET FIBROSIS AND RELEVANT SIGNALING PATHWAYS

Role of PSCs in pancreatic fibrosis

PSCs were identified in the early 1980s through the characteristic of sharing similarities with hepatic stellate cells⁵⁹. PSCs are estimated to constitute 4% of total pancreatic cells, but are essential for maintaining the normal pancreatic architecture by regulating ECM turnover. Quiescent PSCs are characterized by the presence of intracellular fat droplets, desmin and glial

fibrillary acidic protein, but the absence of α -SMA¹⁷. When activated, PSCs are transformed into the myofibroblast-like phenotype characterized by the disappearance of intracellular fat droplets and the expression of α -SMA⁶⁰. The expression of glial fibrillary acidic protein is specific to PSCs in the pancreas, and the presence of lipid droplets in the cytoplasm defines the quiescent phenotype of PSCs. In contrast, the expression of α -SMA represents the transdifferentiation of the quiescent PSCs to an activated phenotype, and thus, is used as a marker of PSC activation⁶¹. Activated PSCs abundantly produce collagen and other ECM proteins, such as fibronectin (Figure 1)¹⁸, and are shown to maintain their activated phenotype through an autocrine loop involving different cytokines. Accordingly, PSCs have been widely researched as the major effector cells in the exocrine pancreatic fibrogenesis, but their role in islet fibrosis has been unclear.

Direct effect of RAS on PSC proliferation and related signaling pathway

Initial studies showed that PSCs were localized to the interlobular and interacinar regions of the pancreas, but not in the islets^{61,62}. In our study on diabetic OLETF rats, α -SMA expression was observed in the peri-islet area of advanced fibrotic islets, not only in the vascular smooth muscle cells and exocrine tissue²⁰. This result was consistent with similar findings of another study of OLETF rats⁶³. We also observed that an ACEi had suppressive effects on ECM protein expression, accompanied by the downregulation of α -SMA²⁰. Overall, PSCs seemed to play a major role in fibrotic islet destruction in animal models of type 2 diabetes mellitus. Nevertheless, it was not clear whether ACE inhibitors directly affected PSCs or had secondary effects through systemic alterations.

At the next step, we isolated PSCs from male Sprague–Dawley rats and cultured PSCs under hyperglycemic conditions²². As a result, PSC proliferation significantly increased at high-glucose concentrations in a dose-dependent manner, up to fourfold, as compared with that under low glucose conditions. The real-time polymerase chain reaction analysis of local-RAS components in PSCs showed that AT1R, angiotensinogen and ACE messenger ribonucleic acids were significantly upregulated in response to increased glucose concentrations, but there was no change in Ang II type 2 receptor level. We also found that TGF- β expression rose after the increase in Ang II levels. Then, the expression of CTGF and type IV collagen proteins increased markedly, and this elevation was effectively attenuated by candesartan and ramipril.

In conclusion, we can explain that Ang II induced by high levels of glucose might stimulate TGF- β synthesis, which subsequently leads to ECM protein synthesis (Figure 2). Furthermore, these results suggest that RAS has a direct impact on PSC proliferation. Thus, ACE inhibitors or ARBs might be effective in preventing or attenuating islet fibrosis in hyperglycemic individuals by directly suppressing PSCs.

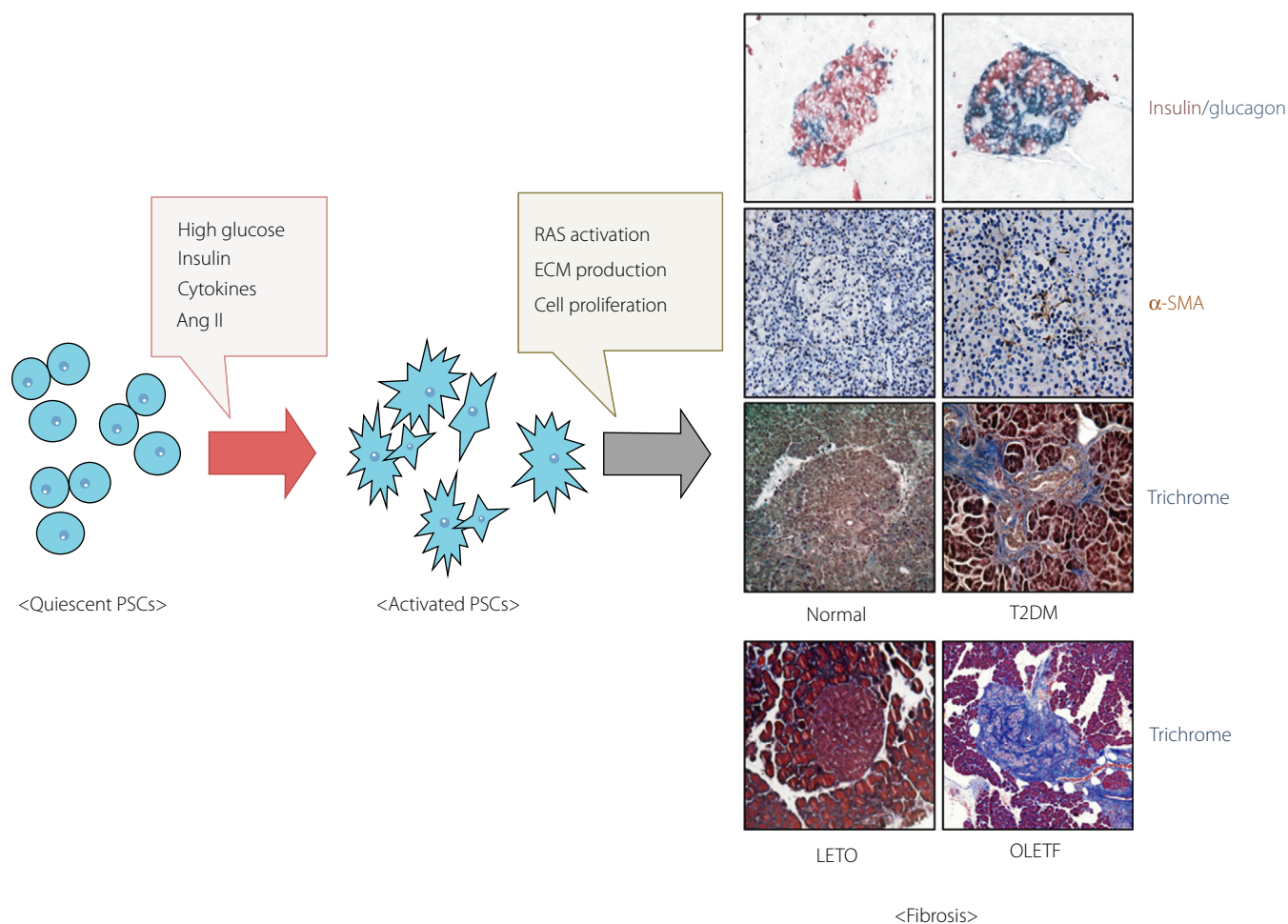


Figure 1 | Pancreatic stellate cell activation and islet fibrosis in type 2 diabetes mellitus (T2DM). High-glucose levels, insulin and angiotensin II (Ang II), and a release of pro-inflammatory cytokines induce pancreatic stellate cell (PSC) activation. Activated PSCs cause extracellular matrix production and cell proliferation. These phenomena appear to drive fibrosis within the pancreatic islets in type 2 diabetes mellitus. Islet fibrosis can be detected by immunostaining for alpha-smooth muscle actin (α -SMA) and trichrome. LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long Evans Tokushima fatty; RAS, renin–angiotensin system.

Additive effects of hyperglycemia and hyperinsulinemia on PSCs: An explanation of the islet-specific fibrosis

Interestingly, a hyperglycemic environment can influence the entire pancreas, but PSCs activation and fibrosis are limited mainly to the islets in the OLETF rat model^{21,63}. Therefore, it remains to be determined why this fibrosis is restricted to islets in individuals with diabetes and animal models of diabetes, even though the whole pancreatic tissue is exposed to hyperglycemia. One possible explanation is that PSCs in the islets might be exposed not only to hyperglycemia, but also to local hyperinsulinemia. Because insulin, a well-known growth factor to various cells in the body⁶⁴, is continuously secreted into the capillaries of the islets at a relatively high concentration, PSCs in islets might be predisposed to activation and proliferation under the influence of hyperinsulinemia. In addition, there is evidence that hepatic stellate cells, functionally similar to PSCs,

are highly sensitive to insulin and insulin-like growth factor-1, resulting in mitogenesis and collagen synthesis⁶⁵.

To confirm this, we stimulated the isolated PSCs with both glucose and insulin, and each⁶⁶. Both stimuli promoted PSCs proliferation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation independently, and the additive effect was also identified. Blocking of ERK signaling by a mitogen-activated protein kinase kinase inhibitor, U0126, inhibited both glucose- and insulin-induced ERK 1/2 phosphorylation and PSC proliferation. In addition, we showed that the glucose- and insulin-induced ERK 1/2 phosphorylation stimulated CTGF gene expression (Figure 2). Thus, we can conclude that hyperglycemia and hyperinsulinemia are two crucial mitogenic factors that activate and proliferate the PSCs, providing a possible explanation for the islet-specific fibrosis in type 2 diabetes mellitus compared with chronic pancreatitis.

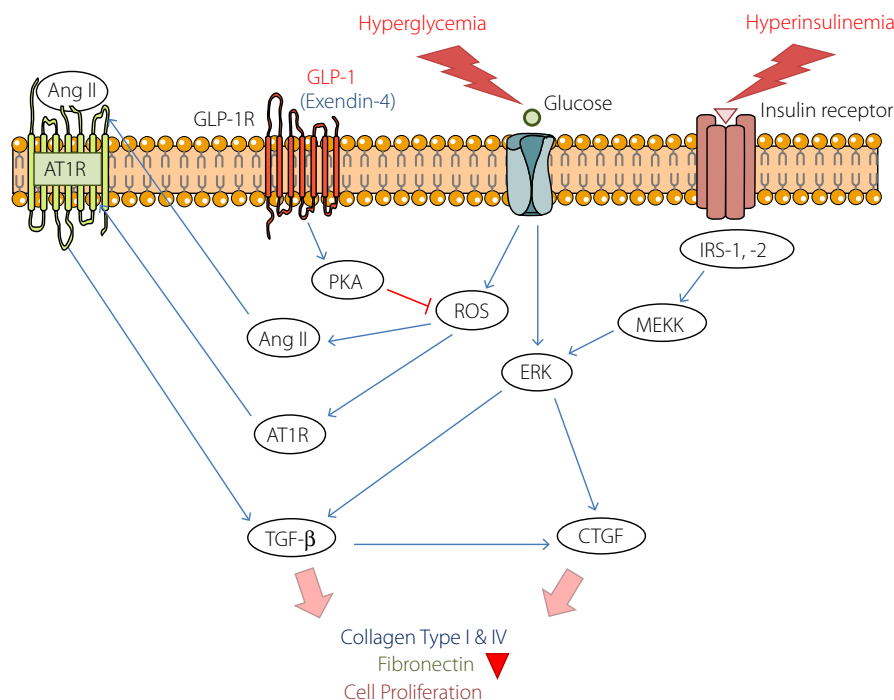


Figure 2 | Mechanism of pancreatic stellate cell activation. Hyperglycemia and hyperinsulinemia: A high-glucose- and insulin-activated pancreatic stellate cell is induced to proliferate through two independent pathways (extracellular signal-regulated kinase [ERK] and angiotensin II [Ang II] pathways). Glucose and insulin independently enhance ERK activation and increase connective tissue growth factor (CTGF) expression. High-glucose concentration stimulates Ang II production and Ang II receptor type 1(AT1R) expression and upregulates transforming growth factor-beta (TGF- β) through the binding of Ang II to AT1R. These pathways ultimately lead to the production of TGF- β 1 and expression of CTGF, an important downstream mediator of TGF- β 1 activity. Finally, these activation events increase collagen and fibronectin formation, and induce cell proliferation. As an antifibrotic effect, the GLP-1 receptor agonist exendin-4 reduces Ang II and TGF- β 1 production through inhibition of protein kinase A (PKA)-related reactive oxygen species (ROS) formation. GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; IRS, insulin receptor substrate; MEKK, mitogen-activated protein kinase kinase.

OTHER POSSIBLE STRATEGIES FOR PREVENTION OF ISLET FIBROSIS

Antidiabetic drugs

Besides using RAS blockers, there have been various other attempts at prevention of islet fibrosis (Table 2). In recent years, GLP-1 analog exendin-4 was shown to improve β -cell function by upregulating key genes involved in insulin secretion⁶⁷. The exendin-4 treatment prevented the development of diabetes in a partial pancreatectomy rat model of type 2 diabetes mellitus, and resulted in a 40% expansion of β -cell mass through the combined effect of differentiation and neogenesis of precursor cells, as well as β -cell proliferation^{68,69}. Furthermore, exendin-4 has been shown to have anti-inflammatory effects, and acts as an antifibrotic agent in mesangial cells^{70,71}. Exendin-4 has been shown to be capable of inhibiting the proliferation of human mesangial cells and downregulating the high-glucose-induced expression of TGF- β 1 and CTGF⁷². Recently, our team reported that exendin-4 significantly reduced Ang II and TGF- β 1 production through inhibition of ROS formation, but not ERK phosphorylation⁷³. These inhibitory effects of exendin-4 were related mainly to the activation of the cyclic adenosine monophosphate-protein kinase A

signaling pathway (Figure 2). Consequently, these data suggest that GLP-1 analogs might be useful not only as of the antidiabetic agent, but also as the antifibrotic therapeutic in type 2 diabetes mellitus.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors, the new oral hypoglycemic agents, function in an insulin-independent manner, and lower blood glucose levels by enhancing urinary glucose excretion. SGLT2 is found to be mainly present in the S1 segment of the proximal renal tubules, and accounts for ~90% of total renal glucose reabsorption. It has been shown that SGLT2 expression in kidneys increases under diabetic conditions⁷⁴. Recently, the SGLT2 inhibitor, dapagliflozin, was found to suppress the expression of components of the renal RAS system and interstitial fibrosis in OLETF rats⁷⁵. Although these effects were confined to the local RAS system and fibrosis in kidneys, it has also been reported that lowering of glucose levels by dapagliflozin attenuates the decline of pancreatic function and the disruption of normal islet morphology in female Zucker diabetic fatty rats⁷⁶.

Nevertheless, it remains unclear how the SGLT2 inhibitor shows protective effects on pancreatic β -cells. Recently, Okouchi *et al.*⁷⁷ reported the effects of another SGLT2 inhibitor,

Table 2 | Possible strategies for prevention of islet fibrosis

Class	Agent	Effects	Reference
Antidiabetic agents	GLP-1 agonist (exendin-4)	Inhibition of ROS production	73
	PPAR- γ agonist (troglitazone)	Reduction of PSC proliferation, Downregulation of TGF- β	94,95
	SGLT2 inhibitor (luseogliflozin)	Downregulation of TGF- β , fibronectin, collagen I and collagen III	77
Antioxidants	Taurine	Downregulation of collagen I and TGF- β	96,97
	Tempol	Downregulation of collagen I and TGF- β	81
	Thioredoxin-1	Attenuation of PSC activation and fibrosis, Downregulation of TGF- β	98
	Ascorbic acid [†]		
Polyphenols	Resveratrol	Inhibition of ROS production	80
	Rhein	Downregulation of collagen I, α -SMA and fibronectin	99
	Emodin	Inhibition of PSC activation	99
	Curcumin	Inhibition of cell proliferation	99,100
	Epigallocatechin-3-gallate	Downregulation of TGF- β	101
Vitamins	Tocotrienols	Inhibition of PSC activation	102
	Retinoic acid	Inhibition of PSC activation, Downregulation of α -SMA and collagen I	103–105
	Palm oil	Downregulation of TGF- β , α -SMA and fibronectin	106
	α -Tocopherol	Attenuation of fibrosis	107
RAS blockers (ACEis & ARBs)	Ramipril	Downregulation of TGF- β	20,22
	Candesartan	Downregulation of TGF- β	22,108,109
	Lisinopril	Downregulation of TGF- β	108,110
	Losartan	Downregulation of TGF- β	111
MEK inhibitors	Trametinib	Downregulation of TGF- β	83
	Dactolisib	Downregulation of α -SMA and collagen I	83
Antifibrotic agents	Pirfenidone	Downregulation of PSC proliferation and collagen I	112

[†]Not yet confirmed. α -SMA, alpha-smooth muscle actin; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GLP-1, glucagon-like peptide-1; MEK, mitogen-activated protein kinase kinase; PPAR- γ , peroxisome proliferator-activated receptor-gamma; PSC, pancreatic stellate cell; RAS, renin-angiotensin system; ROS, reactive oxygen species; SGLT2, sodium-glucose cotransporter 2; TGF- β , transforming growth factor-beta.

luseogliflozin, on β -cell function and mass in the obese *db/db* mouse model of type 2 diabetes mellitus. Luseogliflozin was shown to increase insulin biosynthesis and secretion accompanied by an increased expression of important β -cell-related factors, and was also shown to increase β -cell mass through augmentation of β -cell proliferation and reduction in β -cell apoptosis. Furthermore, the expression levels of fibrosis-related genes, such as TGF- β , fibronectin, collagen I and collagen III, were significantly lower in luseogliflozin-treated animals⁷⁷. These findings suggest that SGLT2 inhibitors might also be promising agents that protect β -cells from islet fibrosis.

Finally, a possible relationship between PPAR- γ agonists and AT1R has been suggested by the finding that activation of PPAR- γ decreases AT1R promoter activity and expression⁷⁸. In addition, the PPAR- γ agonist, troglitazone, has been shown to inhibit the profibrogenic activity of PSCs⁷⁹.

Anti-oxidants, polyphenols and vitamins

One of the other possible therapeutic agents is resveratrol, a natural polyphenolic compound. In a study with *db/db* mice, chronic treatment with resveratrol improved glucose tolerance, attenuated high-glucose-induced oxidative stress, and decreased ROS and islet fibrosis⁸⁰. Taurine and tempol, anti-oxidants,

might also be possible agents that show an antifibrotic effect. They decreased high-glucose-induced PSC activation and islet fibrosis in OLETF rats^{81,82}.

Mitogen-activated protein kinase kinase inhibitors and antifibrotic agents

Mitogen-activated protein kinase kinase inhibitors, which have shown the suppression of both glucose- and insulin-induced ERK 1/2 phosphorylation and PSC proliferation in our previous study, might also be good candidates, although further studies are required to identify their effects^{66,83}. In addition, a recent study on the antifibrotic agent, pirfenidone, showed decreased expression of α -SMA and a smaller extent of islet fibrosis in the pancreas of OLETF rats⁸⁴. However, there was no accompanying improvement in glucose tolerance and insulin secretion⁸⁴. Although that study showed a limited effect, agents that directly target PSCs might also be a targeted strategy either in combination with other drugs or alone.

Targeting of the Mas receptor axis

In addition to the classic RAS pathway, the roles of the Ang II type 2 receptor and the ACE2-Ang (1-7)-Mas receptor axis have been investigated. In a study with high-fat diet C57BL/6

mice, the ACEi, enalapril, enhanced islet remodeling, normalized both α - and β -cell mass, and finally sustained β -cell function⁸⁵. These effects were associated with increased expression of ACE2 and of the MAS receptor, which has the effects opposite to Ang II actions mediated by AT1R^{86,87}. Thus, enhancing the alternative MAS receptor pathway might be a valid strategy to counter islet fibrosis.

CONCLUSION

After substantial clinical evidence, many new and improved insights into the effects of RAS on diabetes have accumulated. Furthermore, new evidence has emerged regarding the involvement of PSCs in endocrine pancreatic function and islet fibrosis. Like antineoplastic agents, aimed at multiple targets to achieve maximal efficacy, the strategies for the prevention of islet fibrosis might offer a benefit by targeting multiple parts in the pathway to disrupt the ominous cross-talk among the related organs. One possible reason why the DREAM trial did not show definitive prevention of diabetes might be that systemic RAS activation was reduced, but the local RAS blockade was incomplete. More potent interventions might, therefore, be required to achieve more definitive results.

In addition, it is important to select appropriate targets according to the risks of patients to attain maximal beneficial effects. Based on the evidence from numerous mega clinical trials, RAS blockers should be considered as the first option to preserve β -cell mass and function in patients with a history of CVD or with cardiovascular risk factors. If individuals have higher levels of systemic inflammation markers, such as elevated serum C-reactive protein levels, an anti-oxidant can be the right choice, although further clinical evidence is required. For patients with prediabetes or with overt type 2 diabetes mellitus, who are thought to have hyperinsulinemia, GLP-1 agonist, SGLT2 inhibitor and PPAR- γ agonist can be good options for slowing the progression of diabetes. In the future, it will be essential to find biomarkers that reflect the status of individual risk factors through studies using genetics and big data. These research advances could provide a more tailored choice of medical treatments based on individual characteristics and thus enabling more specific effects.

DISCLOSURE

The authors declare no conflict of interest.

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