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Oxidative stress plays a role in high glucose-induced activation of pancreatic stellate cells

Gyeong Ryul Ryu [1], Esder Lee [1], Hyun-Ji Chun, Kun-Ho Yoon, Seung-Hyun Ko, Yu-Bae Ahn, Ki-Ho Song *

Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

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ABSTRACT

The activation of pancreatic stellate cells (PSCs) is thought to be a potential mechanism underlying islet fibrosis, which may contribute to progressive β-cell failure in type 2 diabetes. Recently, we demonstrated that antioxidants reduced islet fibrosis in an animal model of type 2 diabetes. However, there is no *in vitro* study demonstrating that high glucose itself can induce oxidative stress in PSCs. Thus, PSCs were isolated and cultured from Sprague Dawley rats, and treated with high glucose for 72 h. High glucose increased the production of reactive oxygen species. When treated with high glucose, freshly isolated PSCs exhibited myofibroblastic transformation. During early culture (passage 1), PSCs treated with high glucose contained an increased number of α-smooth muscle actin-positive cells. During late culture (passages 2–5), PSCs treated with high glucose exhibited increases in cell proliferation, the expression of fibronectin and connective tissue growth factor, release of interleukin-6, transforming growth factor-β and collagen, and cell migration. Finally, the treatment of PSCs with high glucose and antioxidants attenuated these changes. In conclusion, we demonstrated that high glucose increased oxidative stress in primary rat PSCs, thereby facilitating the activation of these cells, while antioxidant treatment attenuated high glucose-induced PSC activation.

1. Introduction

Pancreatic β-cell failure, which is often characterized by a reduced β-cell mass [1,2], plays an important role in the progression of type 2 diabetes [3]. The reduced β-cell mass or β-cell loss in type 2 diabetes is largely attributable to β-cell apoptosis [1], and oxidative stress is an underlying mechanism [4]. Islet fibrosis (the presence of fibrosis in and around the islets) with accompanying islet destruction may be another mechanism of progressive β-cell failure in type 2 diabetes [5–7].

Islet fibrosis is observed in animal models of type 2 diabetes [8–10]. And its presence has been described in patients with type 2 diabetes for many years [11–13]. However, the pathogenesis of islet fibrosis has not been fully elucidated. Islet inflammation or insulitis might be one mechanism. It is hypothesized that prolonged elevation of glucose and free fatty acids might lead to the excessive production of inflammatory cytokines, especially interleukin(IL)-1β, in islets, which induce β-cell apoptosis, islet amyloidosis, and islet fibrosis [14,15]. Another mechanism of islet fibrosis may be the activation of pancreatic stellate cells (PSCs).

PSCs play a critical role in fibrogenesis associated with chronic pancreatitis and pancreatic cancer [16–18]. In these disorders, PSCs transform from a quiescent state into an activated state. During activation, PSCs exhibit a myofibroblastic phenotype, express α-smooth muscle actin (α-SMA) and produce collagen, as well as other extracellular matrix proteins such as fibronectin [19,20]. α-SMA expression is the most commonly used index of PSC activation [19]. Paracrine activators of PSCs include cytokines [IL-1, IL-6, and tumor necrosis factor-α (TNF-α)], growth factors [platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β)], angiotensin II, and reactive oxygen species (ROS) [21]. Once activated, PSCs produce autocrine activators such as PDGF, TGF-β, connective tissue growth factor (CTGF), IL-1, and IL-6 to perpetuate the activation process [18]. Multiple signaling molecules

Abbreviations: PSC, pancreatic stellate cell; IL, interleukin; α-SMA, α-smooth muscle actin; TNF-α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; ROS, reactive oxygen species; CTGF, connective tissue growth factor; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; OLETF, Otsuka Long-Evans Tokushima Fatty; RAS, renin-angiotensin system; PKC, protein kinase C; GBSS, Gey's balanced salt solution; DMEM, Dulbecco's Modified Eagle's Medium; FCS, fetal calf serum; DCF, dichloro-dihydrofluorescein diacetate; BrdU, 5-bromo-2-deoxyuridine; DAPI, 4′,6′-diamidino-2-phenylindole.

* Corresponding author. Address: Department of Internal Medicine, Yeouido St. Mary's Hospital, 62 Yeouido-dong, Youngdeungpo-gu, Seoul 150-713, Republic of Korea. Fax: +82 2 780 3132.
E-mail address: kihos@catholic.ac.kr (K.-H. Song).

[1] These authors contributed equally to this work.

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are involved with PSC activation, including extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase [22].

Interestingly, some reports have suggested that PSCs might be activated in diabetic conditions. Using cultured rat PSCs, we and others have demonstrated that high glucose increased cell proliferation, α-SMA expression, and the production of collagen and fibronectin [23,24]. Moreover, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes, were found to have prominent islet fibrosis, with the presence of α-SMA-positive cells in and around the islets [9,25]. Therefore, PSC activation might be a potential mechanism that underlies islet fibrosis in type 2 diabetes.

However, it remains unclear how a diabetic state or hyperglycemia activates PSCs. One proposed mechanism is the activation of the renin-angiotensin system (RAS). We and others have shown that the blockade of RAS using angiotensin-converting enzyme inhibitors reduced islet fibrosis in OLETF rats and Zucker diabetic fatty rats, another animal model of type 2 diabetes [9,26]. In agreement with these studies, we demonstrated that high glucose increased the angiotensin II levels in cultured rat PSCs [23]. Furthermore, Hama et al. [27] reported that angiotensin II type 1 and type 2 receptors were present in cultured rat PSCs, and that angiotensin II treatment stimulated the proliferation of these cells. Thus, it is plausible that high glucose might activate PSCs via RAS activation. Other mechanisms of high glucose-induced PSC activation have been reported to involve the ERK [28] or protein kinase C (PKC)-p38 MAPK pathway [24].

Oxidative stress is also considered to be a key mediator during PKC activation [22]. Recently, we reported that the treatment of OLETF rats with antioxidants markedly reduced fibrosis and α-SMA expression in the islets, which suggests the involvement of oxidative stress during PSC activation in type 2 diabetes [29]. However, no *in vitro* studies have demonstrated that high glucose itself induces oxidative stress in PSCs. Therefore, we performed the present study to investigate whether high glucose activates PSCs via oxidative stress.

2. Materials and methods

2.1. Isolation and culture of rat PSCs

PSCs were isolated from 14-week-old male Sprague Dawley rats, as reported previously [23]. Briefly, the pancreas was digested in Gey's balanced salt solution (GBSS) containing 1 mg/ml collagenase P (Boehringer-Mannheim, Indianapolis, IN, USA), 0.02% protease (Sigma–Aldrich, St. Louis, MO, USA), and 0.001% DNase I (Boehringer-Mannheim). After passing the digested tissue through a 100 μm mesh, the cells were centrifuged using a 28.7% Nycodenz gradient (Nycomed PharmaAS, Oslo, Norway). The cells were collected at the interface between the Nycodenz and the medium. The cells were then plated in Dulbecco's Modified Eagle's Medium (DMEM) containing 16% fetal calf serum (FCS) and 17.5 mM glucose. This method yielded PSCs with >90% purity and negligible contamination by other cells. The experiments were performed using PSCs between passages 1 and 5 after isolation, except for those that required freshly isolated PSCs.

Unless stated otherwise, PSCs were preincubated in DMEM containing 0.5% FCS and 5.5 mM glucose for 48 h. Next, the cells were treated with low glucose (5.5 mM glucose) or high glucose (30 mM glucose) ± a cocktail of antioxidants [1 mM *N*-acetyl-L-cysteine, 100 μM tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl), and 300 μM taurine (2-amino ethanesulfonic acid)] for 72 h. In some experiments, the cells were also treated with 50 μM H_2O_2.

2.2. Measurement of ROS

To measure the production of ROS, the treated cells were loaded with 5 μM dichloro-dihydrofluorescein diacetate (DCF; Molecular Probes, Eugene, OR, USA) for 30 min at 37 °C. As a positive control, the cells were treated with 50 μM H_2O_2 for 30 min. The cells were washed and analyzed using a scanning fluorometer. The excitation and emission wavelengths were set to 490 and 535 nm, respectively.

2.3. Cell proliferation assay

PSCs were cultured in a 96-well tissue culture plate and treated as described above. Cell proliferation was assessed using a commercial kit [Cell Proliferation ELISA, 5-bromo-2-deoxyuridine (BrdU); Roche Diagnostics, Mannheim, Germany]. After treatment for 24 h, the BrdU uptake rate was determined based on the absorbance at a wavelength of 450 nm.

2.4. Western blot analysis

Western blot analyses were performed as described previously [9]. The primary antibodies were anti-α-SMA antibody (1:2000; Sigma–Aldrich), anti-fibronectin antibody (1:2000; DAKO, Glostrup, Denmark), and anti-CTGF antibody (1:1000; Abcam, Cambridge, MA, USA).

2.5. Immunostaining of α-SMA

The cells were cultured on cover glasses coated with poly-L-lysine. After treatment, the cells were fixed in 4% paraformaldehyde for 10 min and incubated overnight at 4 °C with anti-α-SMA antibody (1:400). The cells were then incubated with rhodamine-labeled anti-mouse IgG antibody (1:100) as the secondary antibody. The nuclei were stained with 4′,6′-diamidino-2-phenylindole (DAPI). The percentage of α-SMA-positive cells was calculated.

2.6. Release of IL-6, TGF-β, or collagen into culture media

The culture media were collected and stored at −80 °C until use. The IL-6 and TGF-β levels were measured using commercial ELISA kits (for IL-6, R&D Systems, Minneapolis, MN; for TGF-β, Promega, Madison, WI, USA). The collagen assay was performed using a commercial kit (Sircol Collagen Assay; Biocolor Ltd, Belfast, UK). Briefly, 1 ml of culture medium was added to 200 μl of cold Isolation and Concentration Reagent, followed by overnight incubation at 4 °C, and centrifugation at 12,000 rpm for 10 min. The concentrated sample was added to 1 ml of Sircol Dye Reagent and centrifuged at 12,000 rpm for 10 min, before 250 μl of Alkali Reagent was added to the pellet. The released dye was read at 540 nm using a microplate reader. The levels of IL-6, TGF-β, and collagen were normalized to the protein content. The protein content was measured using the Bradford method.

2.7. Migration assay

The scratch wound assay [30] was used to measure cell migration. The cells were grown to confluence and a "scratch" was created by scraping the cells in a straight line using a pipette tip. Cell migration was evaluated at 0 and 72 h after the scratch. Images were acquired for each sample and the scratch widths (distance between one side of the scratch and the other) were measured. The migration index was calculated as follows: migration index = (L0−L72)/L0 × 100 (L0 = scratch width at 0 h, L72 = scratch width at 72 h).

2.8. Statistical analysis

Data are expressed as means ± SE. Differences between groups were evaluated using SPSS software (version 10.0; SPSS, Chicago, IL, USA). To analyze the quantitative variables between groups, ANOVA followed by post hoc testing with Fisher's least significant difference test, was used. A P value of <0.05 was considered significant.

3. Results

3.1. ROS generation in PSCs treated with high glucose

To measure ROS generation, PSCs were exposed to DCF, a fluorescent marker of cellular oxidant production [31]. Fig. 1 shows that, similar to H_2O_2, high glucose (30 mM glucose), induced a higher fluorescent intensity as compared with low glucose (5.5 mM glucose), which indicated the induction of oxidative stress by high glucose.

3.2. Activation of PSCs treated with high glucose

Freshly isolated PSCs were incubated in DMEM containing 0.5% FCS and treated with high or low glucose for 72 h. As shown in Fig. 2A, most of the PSCs treated with high glucose transformed into a myofibroblast-like phenotype, whereas PSCs treated with low glucose remained quiescent. During early culture (passage 1), the number of α-SMA-positive cells was higher in PSCs treated with high glucose as compared with those treated with low glucose (Fig. 2B and C). During late culture (passages 2–5), the cell proliferation rate and the release of both IL-6 and collagen increased significantly in PSCs treated with high glucose as compared with those treated with low glucose (Fig. 2C–E). PSCs treated with low glucose + H_2O_2 also exhibited similar responses to those observed in the cells treated with high glucose.

3.3. Effect of antioxidant treatment on the high glucose-induced activation of PSCs

To investigate whether the high glucose-induced facilitation of PSC activation was prevented by antioxidant treatment, we added a combination of antioxidants, i.e., *N*-acetyl-L-cysteine, tempol, and taurine, to PSCs that had been treated with high glucose during late culture. The cell proliferation rate and release of IL-6, TGF-β, and collagen were enhanced significantly in PSCs treated with high glucose. In addition, Western blot analysis detected significant increases in the expression of α-SMA, fibronectin, and CTGF. However, antioxidant treatment attenuated all of these changes remarkably (Fig. 3). Finally, the increased cell migration observed in PSCs with high glucose was also prevented by the antioxidant treatment (Fig. 4).

4. Discussion

The present study showed that high glucose increased ROS production in cultured rat PSCs and facilitated the activation of these cells showing myofibroblastic transformation and increases in the expression of α-SMA, fibronectin, and CTGF, proliferation, release of collagen, IL-6, and TGF-β, and cell migration. However, treatment with antioxidants attenuated these changes.

Animal and human data suggest that islet fibrosis and accompanying islet destruction are present in type 2 diabetes [8–13], although detailed analysis is lacking in humans. Islet fibrosis might appear in the late stage of β-cell dysfunction during the progression to type 2 diabetes [5]. However, the mechanism of islet fibrosis-associated β-cell failure remains obscure. It may accelerate β-cell destruction, as found in chronic pancreatitis [32], or induce the disruption of β-cell connectivity, thereby leading to further impairment of insulin secretion in response to nutrient stimuli [33]. Very recently, PSCs were reported to inhibit β-cell function directly [34].

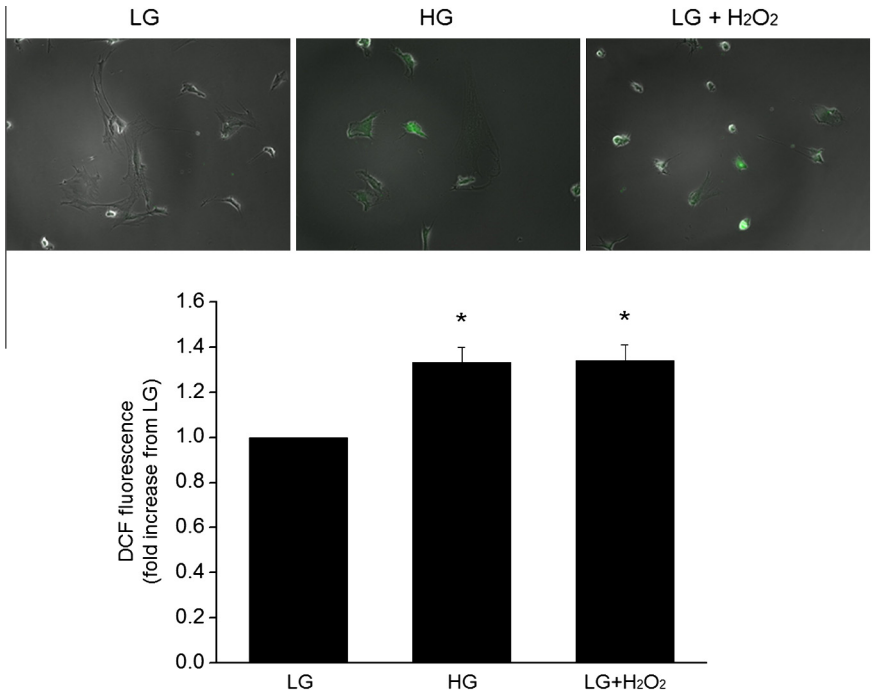

Fig. 1. Generation of ROS. Images showing DCF fluorescence in PSCs after treatment with low glucose (LG), low glucose + H_2O_2 (LG + H_2O_2) or high glucose (HG) for 72 h. DCF fluorescence was quantified using a scanning fluorometer. Magnification 100×. Data are means ± SE (n = 3). *P < 0.05 vs. LG.

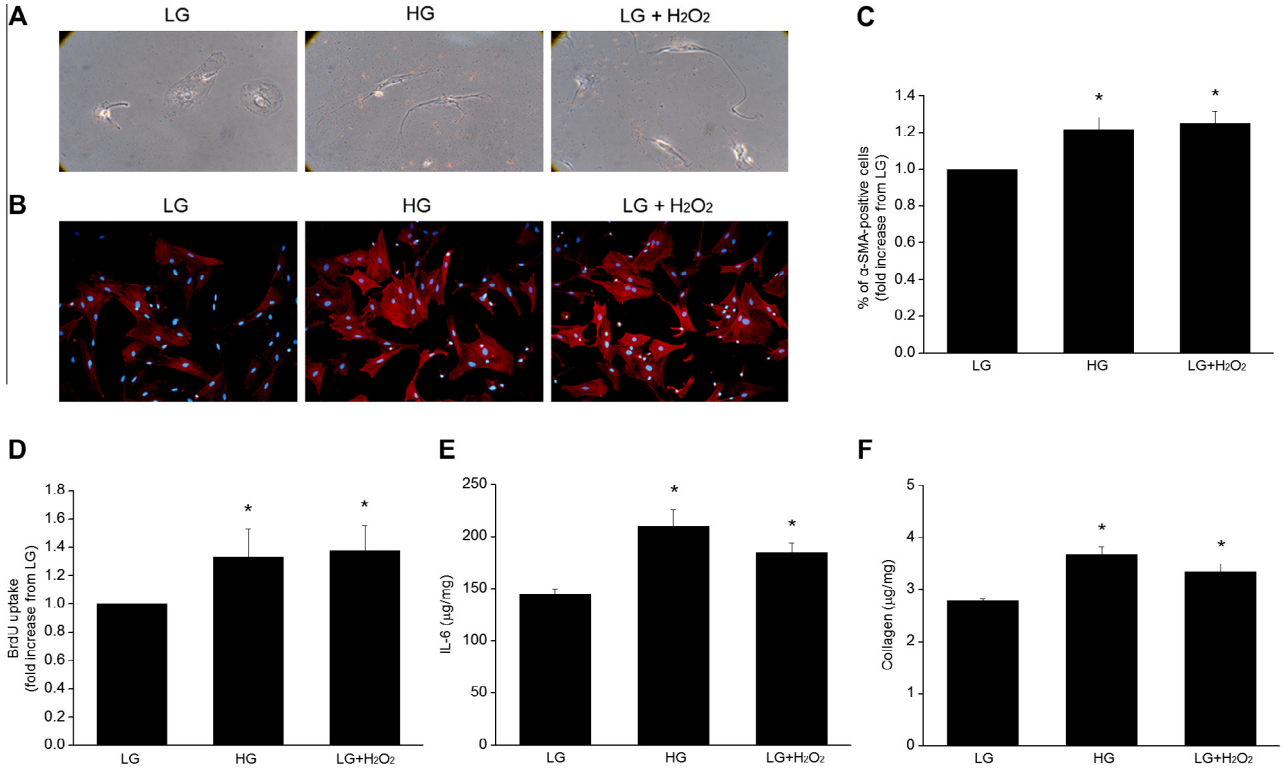

Fig. 2. Activation of PSCs. Images showing the cell morphology in freshly isolated PSCs after treatment with low glucose (LG), low glucose + H_2O_2 (LG + H_2O_2) or high glucose (HG) for 72 h (A). Images showing α-SMA immunostaining (red) in PSCs during early culture (passage 1) after treatment with LG, LG + H_2O_2 or HG for 72 h (B). The nuclei were stained with DAPI (blue). The percentage of α-SMA-positive cells was calculated (C). The BrdU uptake (D) and the release of IL-6 (E) and collagen (F) were measured in PSCs during late culture (passages 2–5) after treatment with LG, LG + H_2O_2 or HG for 72 h. Magnification 200×. Data are means ± SE (n = 5). $^*P < 0.05$ vs. LG.

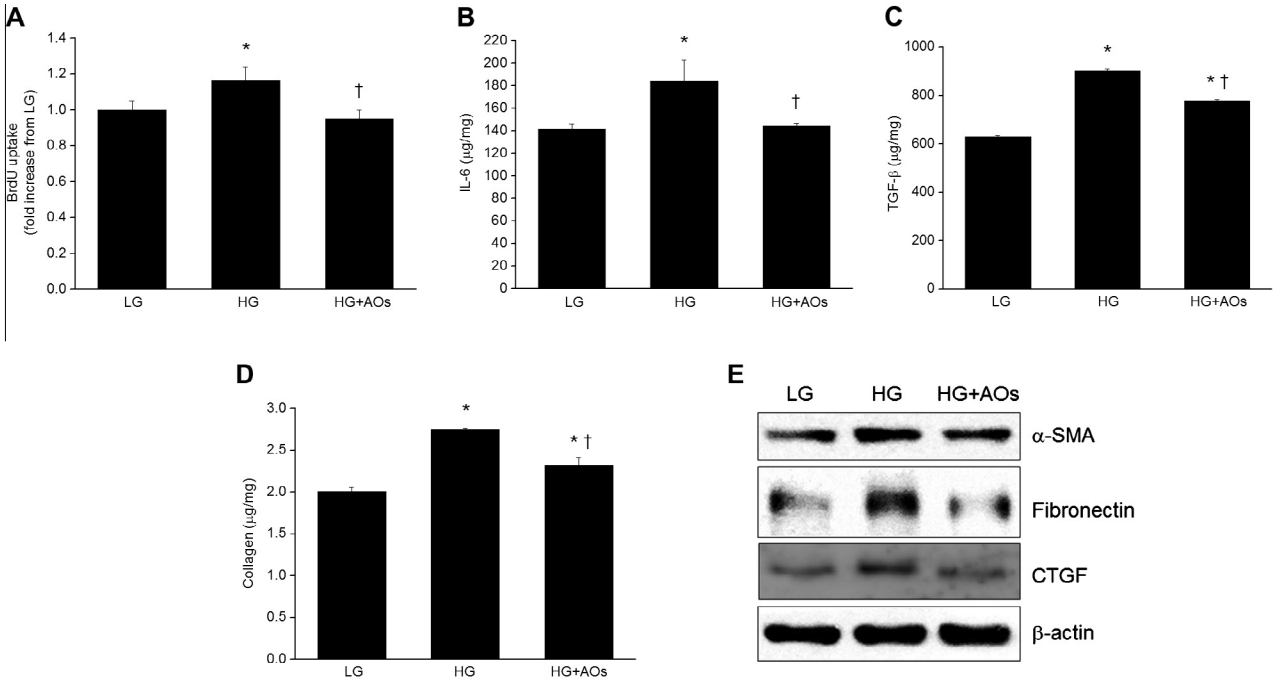

Fig. 3. Effect of antioxidant treatment on the high glucose-induced activation of PSCs. The BrdU uptake (A) and the release of IL-6 (B), TGF-β (C), and collagen (D) were measured in PSCs during late culture (passages 2–5) after treatment with low glucose (LG), high glucose (HG), or high glucose + antioxidants (HG + AOs) for 72 h. The expression of α-SMA, CTGF, and fibronectin were measured by Western blot analysis (E). Data are means ± SE (n = 5). $^*P < 0.05$ vs. LG; †$P < 0.05$ vs. HG.

Thus, it is important to elucidate the development of islet fibrosis to prevent the deterioration of β-cell function in type 2 diabetes. However, its pathogenesis has yet to be clarified. Recent studies have shown that PSCs, which play a critical role in pancreatic fibrosis in chronic pancreatitis and pancreatic cancer [16–18], might also be involved with the development of islet fibrosis in a diabetic

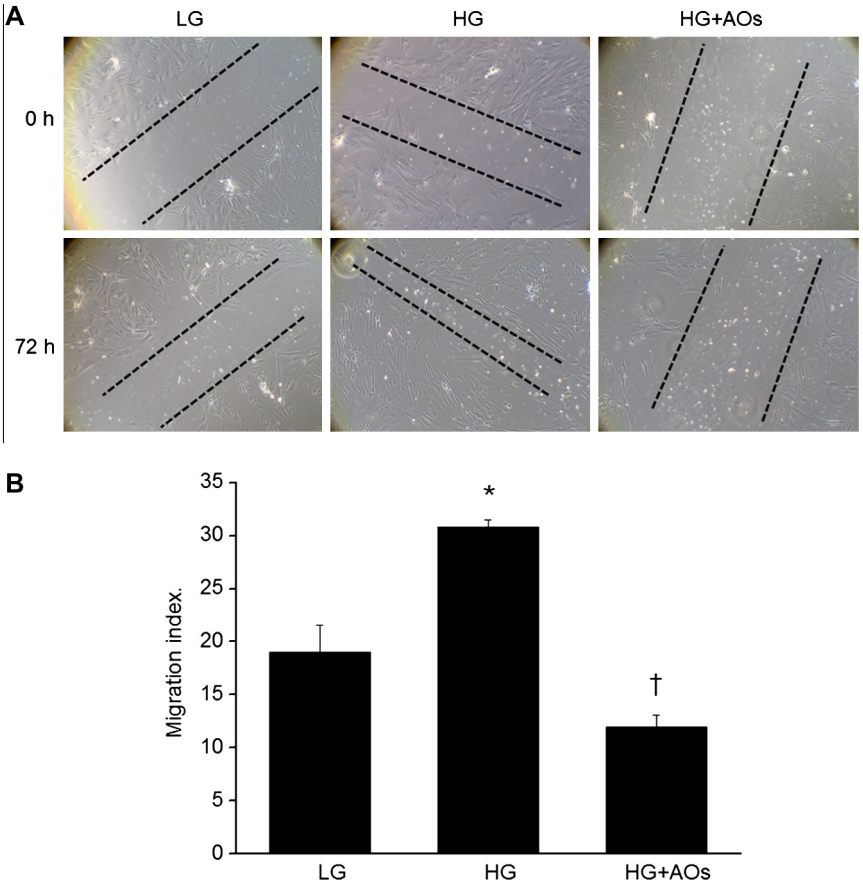

Fig. 4. Analysis of cell migration using the scratch wound assay. Images were acquired at 0 and 72 h after a scratch (A) was made in PSCs during late culture (passages 2–5) following treatment with low glucose (LG), high glucose (HG), or high glucose + antioxidants (HG + AOs) for 72 h. The dotted lines define the areas lacking cells. The migration index was calculated (B). Magnification 200×. Data are means ± SE ($n = 3$). *$P < 0.05$ vs. LG; †$P < 0.05$ vs. HG.

state [9,23–25]. In this study, we focused on the activation of PSCs through high glucose-induced oxidative stress.

It is not surprising that high glucose can induce oxidative stress in PSCs because oxidative stress is well documented in various tissues with chronic hyperglycemia [35]. In islets, β-cells are also a well-known target of oxidative stress [36,37]. And oxidative stress is thought to play an important role in PSC activation [22]. To our knowledge, however, there is no direct evidence confirming that high glucose itself induces oxidative stress in PSCs.

In this study, we demonstrated that high glucose increased the production of ROS in cultured rat PSCs. In agreement with previous studies [23,24], we found that high glucose facilitated PSC activation. First, freshly isolated PSCs (i.e., passage 0) transformed into myofibroblastic phenotypes when treated with high glucose. Second, during early culture (passage 1), PSCs treated with high glucose contained a significantly increased number of α-SMA-positive cells. Third, during late culture (passages 2–5), PSCs treated with high glucose exhibited significant increases in the following: cell proliferation, the expression of CTGF or fibronectin, the release of IL-6, TGF-β, or collagen, and cell migration. H_2O_2 produced similar results to those observed with high glucose, which agreed with previous reports [38,39]. By contrast, other studies have shown that H_2O_2 or 4-hydroxy-2, 3-nonenal, a product of lipid peroxidation, did not initiate the transformation of freshly isolated PSCs into a myofibroblast-like phenotype and they did not affect the proliferation of PSCs during late culture [40,41]. The reason for these discrepancies is unclear, but they may be explained by differences in the cell culture conditions and treatment durations. Finally, these changes were attenuated when high glucose-treated PSCs were exposed to a cocktail of antioxidants. Overall, we showed that high glucose increased the oxidative stress in PSCs, which facilitated the activation of these cells.

In summary, we demonstrated that high glucose increased oxidative stress in primary rat PSCs, thereby facilitating the activation of these cells, while antioxidant treatment attenuated the high glucose-induced PSC activation. Therefore, our data suggest that antioxidant treatment might be beneficial in preventing islet fibrosis and subsequent β-cell failure in patients with type 2 diabetes. However, further studies using human PSCs are required to confirm our findings. In addition, more studies are required to elucidate the molecular mechanism that allows high glucose to induce oxidative stress in PSCs.

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References

[1] A.E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R.A. Rizza, P.C. Butler, Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.

[2] K.H. Yoon, S.H. Ko, J.H. Cho, J.M. Lee, Y.B. Ahn, K.H. Song, S.J. Yoo, M.I. Kang, B.Y. Cha, K.W. Lee, H.Y. Son, S.K. Kang, H.S. Kim, I.K. Lee, S. Bonner-Weir, Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea, J. Clin. Endocrinol. Metab. 88 (2003) 2300–2308.

[3] J.L. Leahy, I.B. Hirsch, K.A. Peterson, D. Schneider, Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 95 (2010) 4206–4216.
[4] G. Lastra, C. Manrique, The expanding role of oxidative stress, renin angiotensin system, and beta-cell dysfunction in the cardiometabolic syndrome and Type 2 diabetes mellitus, Antioxid. Redox Signal. 9 (2007) 943–954.
[5] G.C. Weir, S. Bonner-Weir, Five stages of evolving beta-cell dysfunction during progression to diabetes, Diabetes 53 (Suppl. 3) (2004) S16–21.
[6] J.W. Kim, S.H. Ko, J.H. Cho, C. Sun, O.K. Hong, S.H. Lee, J.H. Kim, K.W. Lee, H.S. Kwon, J.M. Lee, K.H. Song, H.Y. Son, K.H. Yoon, Loss of beta-cells with fibrotic islet destruction in type 2 diabetes mellitus, Front. Biosci. 13 (2008) 6022–6033.
[7] J.H. Cho, J.W. Kim, J.A. Shin, J. Shin, K.H. Yoon, Beta-cell mass in people with type 2 diabetes, J. Diabetes Invest. 2 (2011) 6–17.
[8] A. Pick, J. Clark, C. Kubstrup, W. Pugh, S. Bonner-Weir, K. Polonsky, Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat, Diabetes 47 (1998) 358–364.
[9] S.H. Ko, H.S. Kwon, S.R. Kim, S.D. Moon, Y.B. Ahn, K.H. Song, H.S. Son, B.Y. Cha, K.W. Lee, H.Y. Son, S.K. Kang, C.G. Park, I.K. Lee, K.H. Yoon, Ramipril treatment suppresses islet fibrosis in Otsuka Long-Evans Tokushima fatty rats, Biochem. Biophys. Res. Commun. 316 (2004) 114–122.
[10] F. Homo-Delarche, S. Calderari, J.C. Irminger, M.N. Gangnerau, J. Coulaud, K. Rickenbach, M. Dolz, P. Halban, B. Portha, P. Serradas, Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat, Diabetes 55 (2006) 1625–1633.
[11] W. Gepts, P.M. Lecompte, The pancreatic islets in diabetes, Am. J. Med. 70 (1981) 105–115.
[12] H.L. Zhao, F.M. Lai, P.C. Tong, D.R. Zhong, D. Yang, B. Tomlinson, J.C. Chan, Prevalence and clinicopathological characteristics of islet amyloid in Chinese patients with type 2 diabetes, Diabetes 52 (2003) 2759–2766.
[13] M.R. Hayden, Islet amyloid and fibrosis in the cardiometabolic syndrome and type 2 diabetes mellitus, J. Cardiometab. Syndr. 2 (2007) 70–75.
[14] M. Boni-Schnetzler, J.A. Ehses, M. Faulenbach, M.Y. Donath, Insulitis in type 2 diabetes, Diabetes Obes. Metab. 10 (Suppl 4) (2008) 201–204.
[15] M.Y. Donath, M. Boni-Schnetzler, H. Ellingsgaard, J.A. Ehses, Islet inflammation impairs the pancreatic beta-cell in type 2 diabetes, Physiology (Bethesda) 24 (2009) 325–331.
[16] M.V. Apte, P.S. Haber, T.L. Applegate, I.D. Norton, G.W. McCaughan, M.A. Korsten, R.C. Pirola, J.S. Wilson, Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture, Gut 43 (1998) 128–133.
[17] P.S. Haber, G.W. Keogh, M.V. Apte, C.S. Moran, N.L. Stewart, D.H. Crawford, R.C. Pirola, G.W. McCaughan, G.A. Ramm, J.S. Wilson, Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis, Am. J. Pathol. 155 (1999) 1087–1095.
[18] M. Erkan, G. Adler, M.V. Apte, M.G. Bachem, M. Buchholz, S. Detlefsen, I. Esposito, H. Friess, T.M. Gress, H.J. Habisch, R.F. Hwang, R. Jaster, J. Kleeff, G. Kloppel, C. Kordes, C.D. Logsdon, A. Masamune, C.W. Michalski, J. Oh, P.A. Phillips, M. Pinzani, C. Reiser-Erkan, H. Tsukamoto, J. Wilson, StellaTUM: current consensus and discussion on pancreatic stellate cell research, Gut 61 (2012) 172–178.
[19] M.G. Bachem, E. Schneider, H. Gross, H. Weidenbach, R.M. Schmid, A. Menke, M. Siech, H. Beger, A. Grunert, G. Adler, Identification, culture, and characterization of pancreatic stellate cells in rats and humans, Gastroenterology 115 (1998) 421–432.
[20] M.V. Apte, P.S. Haber, S.J. Darby, S.C. Rodgers, G.W. McCaughan, M.A. Korsten, R.C. Pirola, J.S. Wilson, Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis, Gut 44 (1999) 534–541.
[21] M.B. Omary, A. Lugea, A.W. Lowe, S.J. Pandol, The pancreatic stellate cell: a star on the rise in pancreatic diseases, J. Clin. Invest. 117 (2007) 50–59.
[22] A. Masamune, T. Shimosegawa, Signal transduction in pancreatic stellate cells, J. Gastroenterol. 44 (2009) 249–260.
[23] S.H. Ko, O.K. Hong, J.W. Kim, Y.B. Ahn, K.H. Song, B.Y. Cha, H.Y. Son, M.J. Kim, I.K. Jeong, K.H. Yoon, High glucose increases extracellular matrix production in pancreatic stellate cells by activating the renin-angiotensin system, J. Cell. Biochem. 98 (2006) 343–355.
[24] Y. Nomiyama, M. Tashiro, T. Yamaguchi, S. Watanabe, M. Taguchi, H. Asaumi, H. Nakamura, M. Otsuki, High glucose activates rat pancreatic stellate cells through protein kinase C and p38 mitogen-activated protein kinase pathway, Pancreas 34 (2007) 364–372.
[25] H. Yoshikawa, Y. Kihara, M. Taguchi, T. Yamaguchi, H. Nakamura, M. Otsuki, Role of TGF-beta1 in the development of pancreatic fibrosis in Otsuka Long-Evans Tokushima Fatty rats, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G549–558.
[26] C. Tikellis, P.J. Wookey, R. Candido, S. Andrikopoulos, M.C. Thomas, M.E. Cooper, Improved islet morphology after blockade of the renin-angiotensin system in the ZDF rat, Diabetes 53 (2004) 989–997.
[27] K. Hama, H. Ohnishi, H. Yasuda, N. Ueda, H. Mashima, Y. Satoh, K. Hanatsuka, H. Kita, A. Ohashi, K. Tamada, K. Sugano, Angiotensin II stimulates DNA synthesis of rat pancreatic stellate cells by activating ERK through EGF receptor transactivation, Biochem. Biophys. Res. Commun. 315 (2004) 905–911.
[28] O.K. Hong, S.H. Lee, M. Rhee, S.H. Ko, J.H. Cho, Y.H. Choi, K.H. Song, H.Y. Son, K.H. Yoon, Hyperglycemia and hyperinsulinemia have additive effects on activation and proliferation of pancreatic stellate cells: possible explanation of islet-specific fibrosis in type 2 diabetes mellitus, J. Cell. Biochem. 101 (2007) 665–675.
[29] E. Lee, G.R. Ryu, S.H. Ko, Y.B. Ahn, K.H. Yoon, H. Ha, K.H. Song, Antioxidant treatment may protect pancreatic beta cells through the attenuation of islet fibrosis in an animal model of type 2 diabetes, Biochem. Biophys. Res. Commun. 414 (2011) 397–402.
[30] C.C. Liang, A.Y. Park, J.L. Guan, *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*, Nat. Protoc. 2 (2007) 329–333.
[31] J.A. Royall, H. Ischiropoulos, Evaluation of 2′,7′-dichlorofluorescin and dihydrorhodamine 123 as fluorescent probes for intracellular H_2O_2 in cultured endothelial cells, Arch. Biochem. Biophys. 302 (1993) 348–355.
[32] M. Govindarajan, V. Mohan, R. Deepa, S. Ashok, C.S. Pitchumoni, Histopathology and immunohistochemistry of pancreatic islets in fibrocalculous pancreatic diabetes, Diabetes Res. Clin. Pract. 51 (2001) 29–38.
[33] M.R. Hayden, J.R. Sowers, Isletopathy in Type 2 diabetes mellitus: implications of islet RAS, islet fibrosis, islet amyloid, remodeling, and oxidative stress, Antioxid. Redox Signal. 9 (2007) 891–910.
[34] K. Kikuta, A. Masamune, S. Hamada, T. Takikawa, E. Nakano, T. Shimosegawa, Pancreatic stellate cells reduce insulin expression and induce apoptosis in pancreatic beta-cells, Biochem. Biophys. Res. Commun. 433 (2013) 292–297.
[35] M. Brownlee, The pathobiology of diabetic complications: a unifying mechanism, Diabetes 54 (2005) 1615–1625.
[36] R.P. Robertson, Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes, J. Biol. Chem. 279 (2004) 42351–42354.
[37] H. Kaneto, T.A. Matsuoka, Y. Nakatani, D. Kawamori, T. Miyatsuka, M. Matsuhisa, Y. Yamasaki, Oxidative stress, ER stress, and the JNK pathway in type 2 diabetes, J. Mol. Med. 83 (2005) 429–439.
[38] H. Tanioka, T. Mizushima, A. Shirahige, K. Matsushita, K. Ochi, M. Ichimura, N. Matsumura, T. Shinji, M. Tanimoto, N. Koide, Xanthine oxidase-derived free radicals directly activate rat pancreatic stellate cells, J. Gastroenterol. Hepatol. 21 (2006) 537–544.
[39] A. Masamune, T. Watanabe, K. Kikuta, K. Satoh, T. Shimosegawa, NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells, Am. J. Physiol. Gastrointest. Liver Physiol. 294 (2008) G99–G108.
[40] K. Kikuta, A. Masamune, M. Satoh, N. Suzuki, T. Shimosegawa, 4-hydroxy-2, 3-nonenal activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells, World J. Gastroenterol. 10 (2004) 2344–2351.
[41] K. Kikuta, A. Masamune, M. Satoh, N. Suzuki, K. Satoh, T. Shimosegawa, Hydrogen peroxide activates activator protein-1 and mitogen-activated protein kinases in pancreatic stellate cells, Mol. Cell. Biochem. 291 (2006) 11–20.