Pioglitazone Improves Insulin Secretory Capacity and Prevents the Loss of β-Cell Mass in Obese Diabetic db/db Mice: Possible Protection of β Cells From Oxidative Stress

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In order to assess the beneficial effect of the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist pioglitazone on reduction of mass and alteration of function of pancreatic β cells under diabetic conditions, diabetic C57BL/KsJ db/db mice were treated with pioglitazone for 6 weeks, and insulin secretory capacity and insulin content of isolated pancreatic islets were evaluated. In addition, the expression of oxidative stress markers, 4-hydroxy-2-nonenal (HNE)-modified proteins and heme oxygenase-1, in endocrine pancreas was examined to measure reduction of oxidative stress in pancreatic β cells. The capacity for glucose-induced insulin secretion from isolated islets and their insulin content were improved by pioglitazone treatment ($P < .01$). When β cells were stained with anti-insulin antibodies, those of db/db mice treated with pioglitazone exhibited strong staining, as also observed in their lean littermates. The density of immunostaining for oxidative stress markers was significantly reduced in pancreatic islets of pioglitazone-treated db/db mice ($P < .05$). This study clearly demonstrates the benefit of long-term treatment with pioglitazone in decreasing hyperglycemia and improving glucose-induced insulin secretory capacity in diabetic db/db mice. The results of immunocytochemical examination suggest that this treatment reduces oxidative stress and thereby preserves β-cell mass. Treatment with pioglitazone thus protects against β-cell damage and would be useful for restoration of insulin secretory capacity in obese diabetes individuals.

IT HAS LONG BEEN recognized that insulin resistance in peripheral target tissues and impaired insulin secretory capacity of pancreatic β cells contribute to the pathogenesis of type 2 diabetes.[1-3] Derivatives of thiazolidinedione, which are agonists of nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ), have been shown to enhance insulin sensitivity and to improve metabolic derangements in type 2 diabetes.[4,5] These agents have been reported to prevent the development of diabetes in leptin receptor-deficient Zucker diabetic fatty (ZDF) rats, an animal model of type 2 diabetes.[6] Very recently, it was found that long-term administration of rosiglitazone prevents the loss of β-cell mass in ZDF rats by maintaining β-cell proliferation and preventing increased net β-cell death.[7] It is thus possible that the thiazolidinediones can retard the progression of diabetes under conditions of increased insulin resistance.

db/db mice are known to be inherited the gene encoding the truncated and inactive leptin receptor,[8] and to exhibit severe leptin resistance. This animal model is characterized by hyperphagia, obesity, hyperglycemia, and elevated fasting plasma insulin and closely resembles human type 2 diabetes with peripheral insulin resistance.[9] In addition, it has been demonstrated that PPAR-γ is expressed in pancreatic β cells.[10,11]

In the present study, we assessed the beneficial effect of the PPAR-γ agonist pioglitazone[12,13] on reduction of β-cell mass and alteration of β-cell function under diabetic conditions. Since it has been demonstrated that oxidative stress enhances apoptosis of β cells and suppresses insulin biosynthesis,[14,15] immunostaining of the stress markers 4-hydroxy-2-nonenal (HNE)-modified proteins and heme oxygenase-1 was performed to examine the involvement of oxidative stress in the pathogenesis of β-cell damage and to ascertain the benefits of pioglitazone treatment. The effects of another hypoglycemic agent nateglinide, a short-acting blocker of β-cell adenosine triphosphate (ATP)-sensitive K^+ channels,[16] were compared with those of pioglitazone.

MATERIALS AND METHODS

Animals

Male weanling C57BL/KsJ db/db mice (db/db) and their age-and sex-matched lean littermates (db/+) obtained from Japan Clea (Tokyo, Japan) were raised on standard laboratory chow (Oriental Yeast, Tokyo, Japan). All of the animals were housed in stainless steel cages in an air-conditioned room with a 12-hour light, 12-hour dark cycle in specific pathogen-free conditions. Male db/db mice were divided into 3 groups: an untreated control group, a pioglitazone group (15 mg/kg pioglitazone administered daily through a gastric lavage tube) and a nateglinide group (60 mg/kg nateglinide administered daily by the same method). The administration of vehicle and of these drugs was started at 10 weeks of age, when the diabetic state in db/db mice had already been established. For reference, lean littermates (db/+) were administered vehicle alone and were also examined. The animals were raised in pair-fed state and 3 g of chow was given to each of them twice daily. Blood samples were collected in the fasting state from the tail vein just before treatment, and 2 and 6 weeks after initiation of it. Blood samples were centrifuged at 4°C, and plasma was separated, frozen immediately, and stored at −70°C until assayed. These samples

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Table 1. Fasting Plasma Glucose, Insulin and Triglyceride of the db/db Mice Before and After 2 and 6 Weeks of Treatment

	10 Weeks of Age (before treatment)	12 Weeks of Age (after 2 weeks treatment)	16 Weeks of Age (after 6 weeks treatment)
Fasting plasma glucose (mg/dL, n = 7)			
db/db (untreated)	463.1 ± 19.5	352.0 ± 71.3	321.9 ± 85.0
Pioglitazone	466.4 ± 17.4	118.6 ± 8.4*‡	91.7 ± 7.0†‡
Nateglinide	469.4 ± 17.8	323.1 ± 34.2§	275.5 ± 65.2§
db/+	84.6 ± 3.7*	62.1 ± 3.1*§	97.0 ± 4.9*
Insulin (μU/mL, n = 7)			
db/db (untreated)	177.8 ± 8.0	147.1 ± 26.2	149.8 ± 40.0
Pioglitazone	172.8 ± 48.8	85.0 ± 7.8†‡	66.0 ± 8.4†‡
Nateglinide	147.4 ± 28.8	154.6 ± 30.2	133.3 ± 34.9
db/+	16.4 ± 2.0*	8.4 ± 1.4*§	5.6 ± 0.8*‡
Triglyceride (mg/dL, n = 7)			
db/db (untreated)	311.8 ± 41.9	268.1 ± 21.3	154.6 ± 25.1§
Pioglitazone	288.3 ± 31.9	120.0 ± 12.0*‡	94.1 ± 7.7†‡
Nateglinide	274.3 ± 9.1	260.1 ± 27.0	147.8 ± 19.6§
db/+	78.5 ± 7.1*	66.0 ± 7.5*	65.3 ± 3.2*

*$P < .01$, †$P < .05$ *v* corresponding untreated db/db control.
‡$P < .01$, §$P < .05$ *v* before treatment.

were analyzed for glucose, insulin, and triglyceride levels. Plasma glucose and triglyceride levels were measured using a Hitachi 7350 autoanalyzer (Hitachi, Tokyo, Japan). The homeostasis model assessments (HOMA) index was calculated to estimate peripheral insulin resistance after treatment for 6 weeks with the following formula: fasting plasma glucose (mg/dL) × fasting plasma insulin (μU/mL)/405 (HOMA-R), as described by Matthews et al.[17] The experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The study was approved by the Committee on Animal Care of our university.

Insulin Secretory Capacity of β Cells and Insulin Content of Islets

After treatment for 6 weeks (16 weeks of age), the pancreatic islets were isolated by collagenase digestion and Ficoll gradient centrifugation, as described previously.[18] The islets were picked up under a dissecting microscope. Insulin release from isolated islets was monitored using static incubation. For this purpose, islets were preincubated at 37°C for 1 hour in Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 2.2 mmol/L glucose, 0.2% bovine serum albumin (BSA), and 10 mmol/L HEPES adjusted to pH 7.4. Groups of islets were then batch-incubated for 1 hour in 0.7 mL KRBB containing 2.2 mmol/L, 5.5 mmol/L, 11 mmol/L, and 22 mmol/L glucose. At the end of the incubation periods, islets were pelleted by centrifugation (1,000 × *g*, 180 seconds), and aliquots of the buffer were sampled. The amount of immunoreactive insulin was determined by radioimmunoassay, using rat insulin as a standard, as described previously.[19] Insulin content of the islets was determined at the end of incubation, as previously described.[20]

Histological Studies

The db/db mice of the 3 groups and their lean littermates were used for histological studies. Pancreases were removed, and then fixed with 10% formalin, embedded in paraffin, and sectioned. Aldehyde fuchsin staining was performed. Immunohistochemical staining of β cells using an anti-insulin antibody (Dako, Santa Barbara, CA) was also performed, as previously described.[21] Staining of HNE-modified proteins or heme oxygenase-1 was performed using anti–HNE-modified protein antibody (JICA, Shizuoka, Japan), or anti–heme oxygenase-1 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), respectively, followed by hematoxylin nuclear counterstaining. The RGB-spectrum and contrast of each color image were uniformly adjusted using Adobe Photoshop Version 3.0 (Adobe, San Jose, CA,). The images were then converted to grayscale and analyzed by Scion image software based on NIH image on a Macintosh platform (Scion, Frederick, MD). The HNE- and heme oxygenase-1–positive areas, islet area, and islet cell number were measured after setting a proper threshold on the basis of gray level.

Statistical Analysis

Values are expressed as means ± SE. Statistical analysis was performed with paired and unpaired *t* tests, and analysis of variance (ANOVA) with Fisher's protected least significant difference [PLSD] post hoc test was performed for multiple comparisons. *P* values less than .05 were considered significant.

RESULTS

The body weights of the 3 groups of db/db mice at the beginning of the study did not differ significantly (31.9 ± 0.3 g, 32.6 ± 0.4, and 31.9 ± 0.4 in the untreated control, pioglitazone, and nateglinide groups, respectively), and were all significantly higher than that of the lean littermates (18.1 ± 0.5; $P < .01$). After treatment for 6 weeks, the body weight of the pioglitazone-treated group was 31.2 ± 0.8, and significantly higher ($P < .05$) than those of the other groups, possibly due to its suppression of lipolysis via improvement of insulin resistance (28.4 ± 0.7 and 26.9 ± 0.4, in untreated control and nateglinide groups, respectively), although the value of 18.6 ± 0.4 g in lean littermates was still lower ($P < .01$).

Plasma Glucose, Insulin, and Triglyceride Levels

The changes in fasting plasma glucose, insulin, and triglyceride levels before to after treatment for 2 and 6 weeks are shown in Table 1. Fasting plasma glucose levels were significantly improved in the pioglitazone group after treatment ($P <$ .05 to .01). On the other hand, no significant difference was observed in fasting plasma glucose between the nateglinide and control groups. Plasma insulin in the pioglitazone group was significantly lower than that in the untreated control group after

Table 2. HOMA-R of the db/db Mice Before and After 6 Weeks of Treatment

HOMA-R (× 10, n = 7)	10 Weeks of Age (before treatment)	16 Weeks of Age (after 6 weeks treatment)
db/db (untreated)	20.4 ± 1.1	7.8 ± 1.4‡
Pioglitazone	19.8 ± 2.2	1.6 ± 0.5*‡
Nateglinide	17.5 ± 0.9	7.1 ± 1.3†‡
db/+	0.4 ± 0.1*	0.1 ± 0.2*‡

*$P < .05$ *v* corresponding untreated db/db control.
†$P < .05$ *v* pioglitazone.
‡$P < .01$ *v* before treatment.

treatment ($P < .05$). However, no significant difference was found in this parameter between the nateglinide and control groups. In the untreated control group, plasma triglyceride level was reduced to almost half that before treatment during the observation period. Treatment with pioglitazone further lowered plasma triglyceride level ($P < .05$ to .01), although no significant difference was found in this parameter between the nateglinide and untreated groups.

HOMA-R

To estimate the effects of pioglitazone and nateglinide on insulin resistance in db/db mice, the HOMA-R index was calculated before treatment and after treatment for 6 weeks (Table 2). A higher value of HOMA-R was found in db/db mice before treatment because of severe hyperinsulinemia ($P < .01$). Significant improvement of insulin resistance as measured by HOMA-R was observed after 6 weeks of treatment in all 4 groups ($P < .01$), since they were raised in the pair-fed state (3 g of chow given twice daily) throughout the experiment and were subject to calorie restriction (equal to dietary therapy). Pioglitazone treatment further reduced HOMA-R values in db/db mice ($P < .05$) compared to those in untreated and nateglinide-treated db/db mice, indicating that it can decrease insulin resistance during dietary therapy in the diabetic state.

Insulin Secretory Capacity of β Cells and Insulin Content of Islets

Figure 1 shows the glucose responsiveness of insulin release from islets after treatment for 6 weeks. No significant difference was found among levels of insulin secretion with 2.2 mmol/L glucose (basal insulin release) in the 4 groups. In the lean littermates, insulin secretions with 5.5 mmol/L, 11 mmol/L, and 22 mmol/L glucose were 3.1 ± 1.3 ng/10 islets/h, 18.2 ± 5.0, and 24.2 ± 4.9, respectively, and significantly higher than basal secretion (1.1 ± 0.3, $P < .01$). Insulin secretions by untreated control db/db mice were 0.8 ± 0.4 ng/10 islets/h, 3.1 ± 1.6, and 4.8 ± 1.1 with 5.5 mmol/L, 11 mmol/L, and 22 mmol/L glucose, respectively. These values were significantly lower than those in corresponding lean littermates ($P < .01$). The secretion observed with 5.5 mmol/L glucose was not significantly different from that observed with 2.2 mmol/L glucose (0.8 ± 0.3 ng/10 islets/h), but those observed with 11 mmol/L and 22 mmol/L glucose were significantly higher than that with 5.5 mmol/L glucose ($P < .05$ and $P < .01$, respectively). In the pioglitazone-treated group, insulin secretions with 5.5 mmol/L, 11 mmol/L, and 22 mmol/L glucose were 5.2 ± 1.9 ng/10 islets/h, 8.3 ± 2.1, and 13.4 ± 4.2, respectively, and significantly higher than basal value (0.9 ± 0.3 ng/10 islets/h, $P < .01$). The insulin secretion with

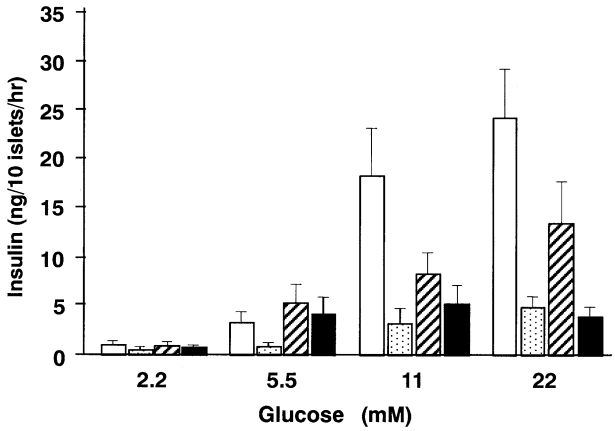

Fig 1. Glucose responsiveness of insulin release from islets after 6 weeks of pioglitazone treatment. In the presence of 2.2 mmol/L glucose (basal insulin release), there were no significant differences among the groups. In the lean littermates (□; db/+), dose-dependent induction of insulin secretion by glucose was observed. The insulin secretion of untreated db/db mice (▨) was significant lower than that of the lean littermates in response to corresponding concentrations of glucose ($P < .01$). In the pioglitazone-treated group (▨), insulin secretion with 5.5 mmol/L, 11 mmol/L, and 22 mmol/L glucose was significantly increased compared to that in the untreated db/db group ($P < .05$ to .01). On the other hand, no additional increase in insulin release was observed in the nateglinide-treated group (■), when islets were stimulated with 11 mmol/L and 22 mmol/L glucose.

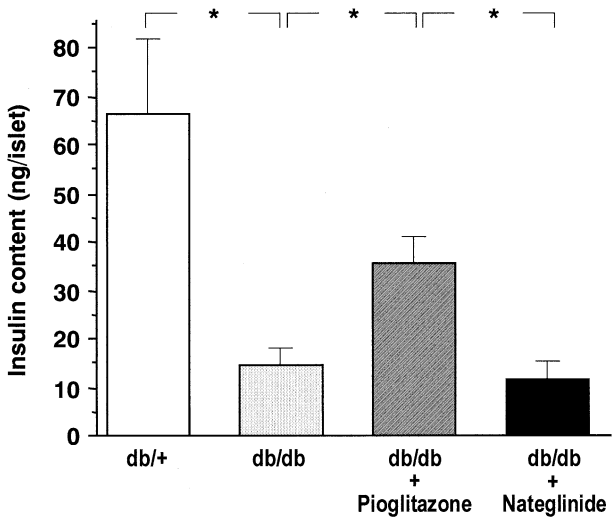

Fig 2. Insulin content of islets after 6 weeks of pioglitazone treatment. In the lean littermates (□; db/+), islet insulin content was significantly higher than in untreated db/db mice (▨). The value in pioglitazone-treated db/db mice (■) was significantly higher than that in untreated db/db mice, but was still lower than that in lean littermates. On the other hand, no significant difference was observed between insulin content in untreated db/db controls and that in nateglinide-treated mice (■), and these values were each significantly lower than that in the pioglitazone-treated group. *$P < .01$.

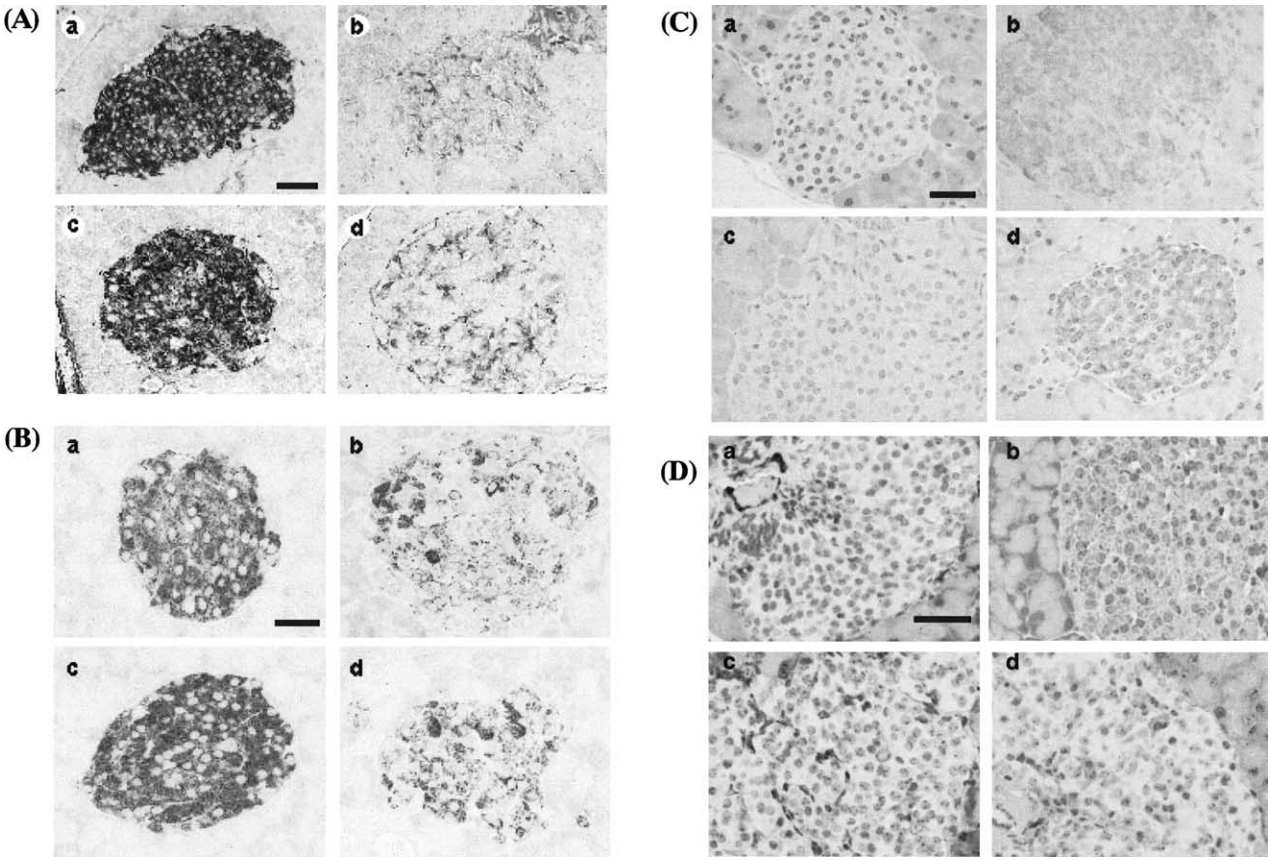

Fig 3. (A) Aldehyde fuchsin staining of β cells. βcell staining of nondiabetic lean littermates is shown in (a). Diabetic db/db mice exhibited degenerated β cells (b), but after long-term treatment with pioglitazone they exhibited active β cells (c). On the other hand, no morphological changes were observed in β cells of nateglinide-treated db/db mice (d), compared to untreated db/db mice. Bar, 20 μm. (B) Immunohistochemical staining for insulin. (b) shows weak staining of β cells with anti-insulin antibodies in untreated db/db mice. db/db mice treated with pioglitazone exhibited strong staining (c), as seen in lean littermates (a). No apparent staining was observed in β cells of db/db mice treated with nateglinide (d). Bar, 20 μm. (C) Expression of HNE-modified proteins was examined by immunostaining. Staining was observed in β cells in untreated db/db mice (b), but was markedly reduced in pioglitazone-treated db/db mice (c). However, staining was still noted in the nateglinide-treated group (d). Bar, 20 μm. (D) Immunostaining of heme oxygenase-1 was observed in β cells in untreated db/db mice (b). This staining was markedly decreased in the pioglitazone-treated group (c). On the other hand, staining was still observed in the nateglinide-treated db/db mice (d). Bar, 20 μm.

5.5 mmol/L glucose was significantly higher than that in untreated db/db mice ($P < .01$). Insulin secretions with 11 mmol/L and 22 mmol/L glucose were significantly higher than those in the corresponding controls ($P < .05$ to .01). Insulin secretion with 5.5 mmol/L glucose in the nateglinide-treated group (4.1 $\pm$ 1.7 ng/10 islets/h) was comparable to that in corresponding lean littermates. However, no additional increase in insulin release was observed in the nateglinide-treated group stimulated with 11 mmol/L and 22 mmol/L glucose (5.1 $\pm$ 2.0 ng/10 islets/h and 3.9 $\pm$ 1.0, respectively).

The insulin content of islets in lean littermates was 66.5 $\pm$ 15.4 ng/islet, and significantly higher than that in untreated control db/db mice (14.8 $\pm$ 3.1, $P < .01$). The value in pioglitazone-treated db/db mice was 35.8 $\pm$ 5.0 ng/islet and significantly higher than that in untreated mice ($P < .01$), as shown in Fig 2. However, insulin content was still lower than that in lean littermates ($P < .01$). On the other hand, there was no difference between the insulin content in nateglinide-treated mice (11.8 $\pm$ 3.7 ng/islet) and that in untreated db/db controls, and the former was significantly lower than that in the pioglitazone-treated group ($P < .01$). These findings suggest that the capacity of islets to synthesize insulin was improved when diabetic db/db/ mice were treated with pioglitazone, but not when they were treated with nateglinide.

Histological Findings

Significant hisitological changes were noted between db/db mice and their lean littermates. On aldehyde fuchsin staining, diabetic db/db mice exhibited hyperplastic pancreatic islets and degenerated β cells, in which degranulation of insulin secretory vesicles had occurred (Fig 3A, b), whereas db/db mice treated with pioglitazone (Fig 3A, c) exhibited hyperplastic islets with active β cells. Treatment with nateglinide did not affect the histology of db/db mice (Fig 3A, d). When β cells were stained with anti-insulin antibodies, those of db/db mice treated with

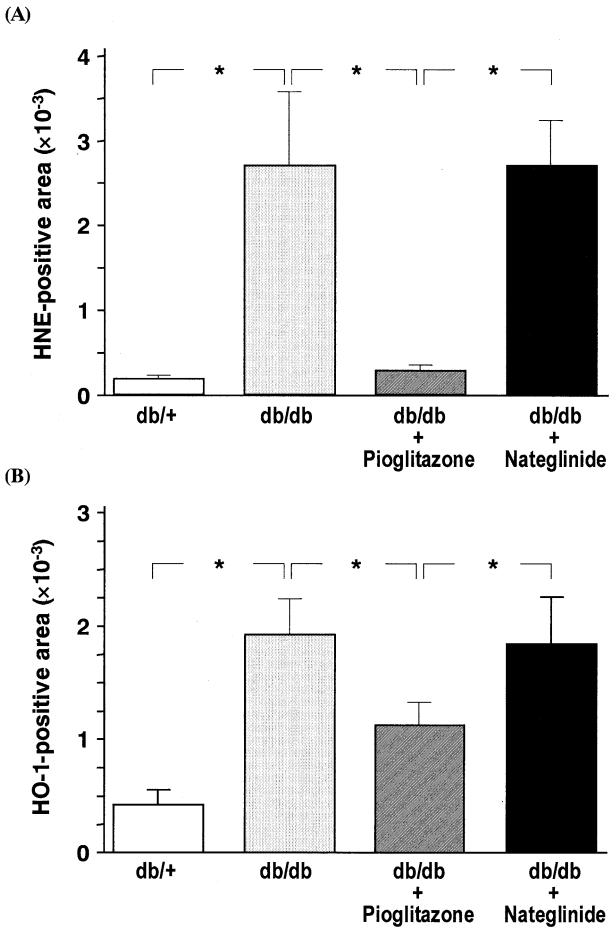

Fig 4. Quantitative analysis of HNE-positive (A) and heme oxygenase-1–positive (B) areas after 6 weeks of treatment. The HNE- and heme oxygenase-1–positive areas (× 10^{-3}), the number of positive islet cells in islet area, were calculated from HNE- and heme oxygenase-1–positive areas (μm^2), respectively. *$P < .05$ (n = 4-7).

pioglitazone exhibited strong staining (Fig 3B, c), as seen in the control littermates (Fig 3B, a). However, no apparent staining of β cells was observed in the nateglinide-treated group (Fig 3B, d). To elucidate the potential benefit of pioglitazone for reduction of oxidative stress in pancreatic β cells, expression of HNE-modified proteins and of heme oxygenase-1 was examined by immunostaining. In untreated db/db mice, staining of these stress markers was observed in the endocrine pancreas (Figs 3C [HNE-modified proteins] and 3D [heme oxygenase-1], b). Staining of these markers was reduced after pioglitazone treatment (Figs 3C and D, c). Staining was, however, still noted in the nateglinide-treated group (Figs 3C and D, d). Quantitative analysis revealed that HNE-positive area was significantly decreased in the pioglitazone-treated db/db mice compared to that in untreated and nateglinide-treated db/db mice ($P < .05$; Fig 4A). The density of staining of heme oxygenase-1 was also significantly reduced in the pioglitazone-treated group ($P < .05$; Fig 4B). These results clearly indicate that treatment with pioglitazone efficiently reduces the oxidative stress induced in endocrine pancreas of diabetic db/db mice.

DISCUSSION

This study clearly demonstrated that long-term treatment with the insulin sensitizer pioglitazone ameliorates hyperglycemia and hypertriglyceridemia in obese diabetic db/db mice. In addition, immunocytochemical examination revealed that treatment with pioglitazone can prevent loss of β-cell mass. These results agree with the recent finding that long-term administration of rosiglitazone, an thiazolidinedione derivative, prevented disruption of pancreatic islet architecture in ZDF rats.[7] The batch incubation experiment with pancreatic islets of db/db mice directly demonstrated beneficial effects of pioglitazone on insulin content of islets and glucose-induced insulin secretory capacity. On the other hand, since the mechanism by which nateglinide induces hypoglycemia does not involve reduction of peripheral insulin resistance,[16] this agent failed to improve the metabolic derangements and morphological alterations of islets in db/db mice.

Prolonged hyperglycemia has been considered to be toxic for pancreatic β cells. Because prolonged exposure to high glucose concentrations impairs the glucose responsiveness of insulin release from β cells,[22,23] the restoration of glucose-induced secretory capacity after pioglitazone treatment is thought to be due to elimination of glucotoxicity. Another possible explanation for this restoration could be the long-term reduction of insulin resistance by pioglitazone in vivo. Improvement of peripheral insulin sensitivity through PPAR-γ activation would avoid excessive insulin release and insulin content of the islets would be preserved, resulting in restoration of insulin secretory capacity. On the other hand, recent studies have revealed that high glucose concentrations induced a process of apoptotic β-cell death[24,25] and that oxidative stress induced by chronic hyperglycemia reduced β-cell mass due to apoptosis.[14,15] Interestingly, our immunostaining examination indicated that oxidative stress was markedly reduced in pancreatic islets after pioglitazone treatment and that loss of β-cell mass was eventually prevented by this agent.

It has been reported that troglitazone, another thiazolidinedione derivative, improves hyperlipidemia and exerts direct lipopenic effects in islets while improving β-cell function of obese prediabetic ZDF rats.[26] We recently observed that high fatty acid levels suppress glucose-induced proinsulin biosynthesis.[27] The preservation of insulin content after long-term pioglitazone treatment might therefore be due at least in part to improvement of hyperlipidemia. In addition, the reduction of oxidative stress in db/db islets could be due to relief of hyperlipidemia, since incubation in the presence of elevated concentrations of fatty acids induces β-cell death through apoptosis.[28] However, since hyperlipidemia has been reported to be deleterious only in the presence of hyperglycemia,[29,30] the beneficial effects observed with pioglitazone treatment appear to be mainly due to its dramatic reduction of plasma glucose levels.

The accepted classical concept of insulin resistance is that it is due to disturbance of the intracellular signaling pathway in peripheral tissues expressing functional insulin receptors, such as muscle, white adipose tissue, and liver. However, in addition to PPAR-γ, it has recently been found that the insulin receptor and its substrate proteins are also functionally expressed in insulin-secreting pancreatic β cells.[31-33] In fact, following tis-

sue-specific knockout of the insulin receptor in β cells (β IRKO), impairment of glucose-induced insulin secretion and basal hyperinsulinemia has been observed along with deterioration of glucose tolerance.[34] It has therefore been suggested that chronic defects in insulin signaling at the β-cell level may be involved in the pathogenesis of type 2 diabetes. In this context, the beneficial effect of long-term pioglitazone treatment could accordingly be due to amelioration of defective insulin signaling in diabetic β cells. Upregulation by PPAR-γ of glucokinase gene expression in β cells, as recently demonstrated,[35] should contribute to the restoration of glucose-induced insulin secretory capacity. On the other hand, it has been reported that tumor necrosis factor (TNF)-α from adipocytes, a cytokine proposed to mediate insulin resistance in insulin target tissues by interfering with insulin signaling, similarly induces insulin resistance in pancreatic β cells,[36] a state characterized by disturbance of intracellular metabolism. The decrease of TNF-α expression by pioglitazone in adipose tissue in db/db mice[37] might result in improvement of β-cell insulin resistance. Interestingly, it has recently been reported that this agent can reduce oxidative stress, and that it contributes to reduction of susceptibility of low-density lipoproteins to oxidation as well as decrease in aortic wall stiffness in diabetic animals.[38,39] Further detailed study, such as measurement of the induction of anti-oxidant enzyme activities in β cells, is needed to clarify the mechanism of reduction of oxidative stress by which β-cell function is improved by pioglitazone treatment.

In conclusion, the present study demonstrated that treatment with pioglitazone restores the insulin secretory capacity of pancreatic β cells of obese diabetic db/db mice. It seems likely that this beneficial effect is due in part to prevention of loss of β-cell mass through reduction of oxidative stress. The precise mechanism of this effect remains to be elucidated.

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REFERENCES

1. Martin BC, Warram JH, Krolewski AS, et al: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: Results of a 25-year follow-up study. Lancet 340:925-929, 1992

2. Lillioja S, Mott DM, Spraul M, et al: Insulin resistance and insulin secretory dysfuncton as precursors of non-insulin-dependent diabetes mellitus: Prospective studies of Pima Indians. N Engl J Med 329:1988-1992, 1993

3. Polonsky KS, Sturis J, Bell GI: Non-insulin-dependent diabetes mellitus: A genetically programmed failure of the beta cell to compensate for insulin resistance. N Engl J Med 334:777-783, 1996

4. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ. J Biol Chem 270:12953-12956, 1995

5. Berger J, Bailey P, Biswas C, et al: Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor γ: Binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137:4189-4195, 1996

6. Higa M, Zhou YT, Ravazzola M, et al: Troglitazone prevents mitochondrial alterations, beta cell destruction and diabetes in obese prediabetic rats. Proc Natl Sci USA 96:11513-11518, 1999

7. Finegood DT, McArthur MD, Kojwan D, et al: β cell mass dynamics in Zucker diabetic fatty rats: Rosiglitazone prevents the rise in net cell death. Diabetes 50:1021-1029, 2001

8. Chua SC Jr, Chung WK, Wu-Peng XS, et al: Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271:994-996, 1996

9. Ishida H, Cunningham NS, Henry HL, et al: The number of 1,25-dihydroxyvitamin D3 receptors is decreased in both intestine and kidney of genetically diabetic db/db mice. Endocrinology 122:2436-2443, 1988

10. Zhou Y-T, Shimabukuro M, Wang M-Y, et al: Role of peroxisome proliferator-activated receptor α in disease of pancreatic β cells. Proc Natl Acad Sci USA 95:8898-8903, 1998

11. Unger RH, Zhou Y-T, Orci L: Lipotoxicity, in LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus. A Fundamental and Clinical Text (ed 2). Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 132-141

12. Sugiyama Y, Taketomi S, Shimura Y, et al: Effect of pioglitazone on glucose and lipid metabolism in Wistar fatty rats. Arzneimforsch Drug Res 40:263-267, 1990

13. Hayakawa T, Shiraki T, Morimoto T, et al: Pioglitazone improves insulin signaling defects in skeletal muscle from Wistar fatty (fa/fa) rats. Biochem Biophys Res Commun 223:439-444, 1996

14. Tanaka Y, Cleason CE, Tran PO, et al: Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. Proc Natl Acad Sci USA 96:10857-10862, 1999

15. Kaneto H, Kajimoto Y, Miyagawa J, et al: Beneficial effects of antioxidants in diabetes: Possible protection of pancreatic β-cells against glucose toxicity. Diabetes 48:2398-2406, 1999

16. Hu S, Wang S, Fanelli B, et al: Pancreatic β-cell KATP channel activity and membrane-binding studies with nateglinide: A comparison with sulfonylureas and repaglinide. J Pharmacol Exp Ther 293:444-452, 2000

17. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985

18. Nagamatsu S, Nakamichi Y, Yamamura C, et al: Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreatic β-cells is involved in impaired insulin secretion from diabetic GK rat islets: Restoration of decreased t-SNARE proteins improves impaired insulin secretion. Diabetes 48:2367-2373, 1999

19. Fujimoto S, Ishida H, Kato S, et al: The novel insulinotropic mechanism of pimobendan: Direct enhancement of the exocytotic process of insulin secretory granules by increased Ca^{2+} sensitivity in β-cells. Endocrinology 139:1133-1140, 1998

20. Tsuji K, Taminato T, Usami M, et al: Characteristic features of insulin secretion in the streptozotocin-induced NIDDM rat model. Metabolism 37:1040-1044, 1998

21. Than S, Ishida H, Inaba M, et al: Bone marrow transplantation as a strategy for treatment of non-insulin-dependent diabetes mellitus in KK-Ay mice. J Exp Med 176:1233-1238, 1992

22. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: A balanced overview. Diabetes Care 15:318-368, 1992

23. Yki-Jarvinen H: Glucose toxicity. Endocrine Rev 13:415-431, 1992

24. Efanova IB, Zaitsev SV, Zhivotovsky B, et al: Glucose and tolbutamide induce apoptosis in pancreatic β-cells: A process dependent on intracellular Ca^{2+} concentration. J Biol Chem 273:33501-33507, 1998

25. Liu K, Paterson AJ, Chin E, et al: Glucose stimulates protein modification by O-linked GlcNAc in pancreatic β cells: Linkage of

O-linked GlcNAc to β cell death. Proc Natl Acad Sci USA 97:2820-2825, 2000

26. Shimabukuro M, Zhou Y-T, Lee Y, et al: Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. J Biol Chem 273:3547-3550, 1998

27. Katahira H, Nagamatsu S, Ozawa S, et al: Acute inhibition of proinsulin biosynthesis at the translational level by palmitic acid. Biochem Biophys Res Commun 282:507-510, 2001

28. Shimabukuro M, Zhou Y-T, Levi M, et al: Fatty acid-induced β cell apoptosis: A link between obesity and diabetes. Proc Natl Acad Sci USA 95:2498-2502, 1998

29. Poitout V, Robertson RP: Minireview: Secondary β cell failure in type 2 diabetes—A convergence of glucotoxicity and lipotoxicity. Endocrionology 143:339-342, 2002

30. Harmon JS, Gleason CE, Tanaka Y, et al: Antecedent hyperglycemia, not hyperlipidemia, is associated with increased islet triacylglycenol content and decreased insulin gene mRNA levels in Zucker diabetic fatty rats. Diabetes 50:2481-2486, 2001

31. Harbeck MC, Louie DC, Howland J, et al: Expression of insulin receptor mRNA and insulin receptor substrate 1 in pancreatic islet β-cells. Diabetes 45:711-717, 1996

32. Xu GG, Rothenberg PL: Insulin receptor signaling in the β-cell influences insulin gene expression and insulin content: Evidence for autocrine β-cell regulation. Diabetes 47:1243-1252, 1998

33. White MF, Withers D: Insulin receptor substrate proteins mediate common signaling pathways for insulin action and β-cell function, in LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus. A Fundamental and Clinical Text (ed 2). Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 199-206

34. Kulkarni RN, Brüning JC, Winnay JN, et al: Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96:329-339, 1999

35. Kim H-i, Cha J-Y, Kim S-Y, et al: Peroxisomal proliferator-activated receptor-γ upregulates glucokinase gene expression in β cells. Diabetes 51:676-685, 2002

36. Kwon G, Xu G, Marshall CA, et al: Tumor necrosis factor α-induced pancreatic β-cell insulin resistance is mediated by nitric oxide and prevented by 15-deoxy-Δ12,14-prostaglandin J2 and aminoguanidine. J Biol Chem 274:18702-18708, 1999

37. Maeda N, Takahashi M, Funahashi T, et al: PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094-2099, 2001

38. Iida KT, Kawakami Y, Suzuki M, et al: Effect of thiazolidinediones and metformin on LDL oxidation and aortic endothelium relaxation in diabetic GK rats. Am J Physiol 284:E1125-E1130, 2003

39. Tsuji T, Mizushige K, Noma T, et al: Improvement of aortic wall distensibility and reduction of oxidative stress by pioglitazone in pre-diabetic stage of Otsuka Long-Evans Tokushima fatty rats. Cardiovasc Drugs Ther 16:429-434, 2002