Imatinib Mesylate Reduces Endoplasmic Reticulum Stress and Induces Remission of Diabetes in *db/db* Mice

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OBJECTIVE—Imatinib has been reported to induce regression of type 2 diabetes in chronic leukemia patients. However, the mechanism of diabetes amelioration by imatinib is unknown, and it is uncertain whether imatinib has effects on type 2 diabetes itself without other confounding diseases like leukemia. We studied the effect of imatinib on diabetes in *db/db* mice and investigated possible mechanism's underlying improved glycemic control by imatinib.

RESEARCH DESIGN AND METHODS—Glucose tolerance and insulin tolerance tests were done after daily intraperitoneal injection of 25 mg/kg imatinib into *db/db* and C57BL/6 mice for 4 weeks. Insulin signaling and endoplasmic reticulum stress responses were studied by Western blotting. β-Cell mass and apoptotic β-cell number were determined by combined terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining and insulin immunohistochemistry. The in vitro effect of imatinib was studied using HepG2 cells.

RESULTS—Imatinib induced remission of diabetes in *db/db* mice and amelioration of insulin resistance. Expression of endoplasmic reticulum stress markers in the liver and adipose tissues of *db/db* mice, such as phospho-PERK, phospho-eIF2α, TRB3, CHOP, and phospho–c-Jun NH_2-terminal kinase, was reduced by imatinib. Insulin receptor substrate-1 tyrosine phosphorylation and Akt phosphorylation after insulin administration were improved by imatinib. Serum aminotransferase levels and hepatic triglyceride contents were decreased by imatinib. Pancreatic β-cell mass was increased by imatinib, accompanied by decreased $TUNEL^+$ β-cell and increased $BrdU^+$ β-cell numbers. Imatinib attenuated endoplasmic reticulum stress in hepatoma cells in vitro.

CONCLUSIONS—Imatinib ameliorated endoplasmic reticulum stress and induced remission of diabetes in *db/db* mice. Imatinib or related compounds could be used as therapeutic agents against type 2 diabetes and metabolic syndrome. ***Diabetes* 58: 329–336, 2009**

Type 2 diabetes is a metabolic disease that affects 2.8% of all age-groups worldwide, and this proportion is expected to increase to 4.4% by 2030 (1). The two major components of the pathogenesis of type 2 diabetes are insulin resistance and β-cell failure. However, the biochemical mechanisms underlying these two phenomena are incompletely understood.

Regarding the mechanism of insulin resistance, several hypotheses have been proposed, for example, increased circulating free fatty acid level, mitochondrial dysfunction, elevated reactive oxygen species production, and increased levels of proinflammatory mediators (2–4). Downstream of these molecules or events, disturbance in intracellular signaling, such as c-Jun NH_2-terminal kinase (JNK) phosphorylation, IKKβ activation, or endoplasmic reticulum stress responses, may play a role in the development of insulin resistance (5–7).

Imatinib mesylate (Gleevec) is a well-known anticancer agent that has a dramatic effect on chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor by specifically inhibiting Bcr-Abl or Kit kinase (8,9). Recently, it was reported that imatinib induced remission of type 2 diabetes in patients having both CML and type 2 diabetes (10,11). The effect of imatinib on animal models of type 1 diabetes resulting from islet injury has also been studied (12,13). However, the effect of imatinib on type 2 diabetes itself in patients or experimental animals without other confounding diseases has not been explored. Furthermore, the mechanism of the improvement of type 2 diabetes by imatinib is unknown. In fact, the possibility that improved glucose tolerance in type 2 diabetic patients with CML by imatinib is due to a positive side effect of leukemic responses leading to the nonspecific generalized improvement of health status cannot be eliminated (11). Thus, it is uncertain whether imatinib could improve glycemic control in type 2 diabetic patients or animals without other confounding diseases. To address this question, we investigated whether imatinib could improve glycemic control in diabetic *db/db* mice.

RESEARCH DESIGN AND METHODS

Cell line and reagents. HepG2 hepatoma cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium–10% fetal bovine serum containing 2 mmol/l glutamine and penicillin-streptomycin (Gibco, Carlsbad, CA). All other chemicals were obtained from Sigma (St. Louis, MO), unless stated otherwise.

In vivo models. Imatinib (provided by Dr. R. Halse, Novartis Pharma, Basel, Switzerland) was dissolved fresh in PBS and injected intraperitoneally into *db/db* (The Jackson Laboratory, Bar Harbor, ME) and control C57BL/6 mice. Control littermate *db/db* mice were treated with PBS. Intraperitoneal glucose tolerance test (IPGTT) was carried out after overnight fasting by intraperitoneal injection of 1 g/kg glucose (14). Blood glucose concentrations were determined using a One Touch glucometer (Lifescan, Milpitas, CA) before (0

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min) and 15, 30, 60, and 120 min after glucose injection. Insulin tolerance test (ITT) was conducted by intraperitoneal injection of 1 unit/kg regular insulin to fasted mice and measuring blood glucose levels at 0, 15, 30, 60, and 120 min. Insulinogenic index was calculated according to a published method ($\Delta insulin_{15min}/\Delta glucose_{15min}$) (15,16). Serum insulin concentrations were determined using a commercial mouse insulin ELISA kit (Shibayagi, Gunma, Japan). Pair feeding was conducted as previously described (17). To examine insulin signaling in vivo, regular insulin (5 units/kg) was injected into the tail vein. Five minutes after insulin infusion, the liver and epididymal adipose tissues were removed and frozen in liquid nitrogen until use. Alanine aminotransferase/aspartate aminotransferase (ALT/AST) levels were measured using a Fuji Dri-Chem 3000 blood chemistry analyzer (Fuji Photo, Tokyo, Japan) according to the manufacturer's instruction. All animal experiments were conducted in accordance with the institutional guidelines of Samsung Medical Center Animal Facility.

Western blotting. Western blotting was as previously described (18) using anti–phospho-JNK Thr183/Tyr185, anti–insulin receptor substrate-1 (anti–IRS-1), anti–phospho-eukaryotic initiation factor 2a (elF2α) Ser51, anti–phospho-double-stranded RNA-activated protein-like endoplasmic reticulum kinase (PERK) Thr980, anti-Akt, anti–phospho-Akt Thr308 (Cell Signaling, Beverly, MA), anti–phospho-IRS-1 Ser307 (Upstate Biotechnology, Lake Placid, NY), anti–phospho-IRS-1 Tyr612 (Biosource International, Camarillo, CA), anti-GADD153/C/EBP homologous protein (CHOP) (Sigma), anti-elF2α, anti-JNK (Santa Cruz Biotechnology, Santa Cruz, CA), anti–β-actin (Abcam, Cambridge, MA), and anti-TRB3 (provided Dr. S.H. Koo, Sungkyunkwan University School of Medicine) antibodies.

Triglyceride measurement. Lipid was extracted from homogenized tissue using a 2:1 chloroform:methanol mixture. Lipid residue after evaporation was suspended in 1% Triton X-100 in 100% ethanol and mixed with Free Glycerol Reagent containing lipase (Sigma). After incubation at 37°C for 5 min, A_{540} was measured for calculation of triglyceride (TG) concentrations on a standard curve.

β-Cell mass. To measure pancreatic β-cell mass, formalin-fixed sections of the pancreas were deparaffinized and briefly microwaved in 0.01 mol/l sodium citrate buffer (pH 6.0). They were then incubated with an appropriate dilution of anti-insulin antibody (DAKO, Kyoto, Japan) after goat serum blocking. Incubation with biotinylated anti–guinea pig IgG antibody and then with avidin-biotin-peroxidase complex (Vector, Burlingame, CA) followed. Diaminobenzidine was used as a color substrate. Point-counting morphometry on insulin antibody-stained sections was done to calculate the relative β-cell area (19) Terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining combined with immunohistochemistry was conducted to identify apoptotic β-cells in pancreatic sections as described previously (18). To quantify apoptotic β-cells, >50 islets from more than three parallel sections obtained at different cut levels were analyzed per mouse by a single observer, unaware of mouse genotype. To quantify β-cell proliferation, 100 μg/g 5-bromo-2′-deoxyuridine (BrdU) (Serva, Heidelberg, Germany) in 5 mmol/l NaOH-PBS was injected intraperitoneally. Pancreatic sections prepared 12 h after BrdU injection were treated with 4.2 N HCl in PBS and then stained with anti-BrdU antibody. After immunohistochemistry using anti-insulin antibody as the primary antibody, numbers of $BrdU^+$ β-cells were calculated as described above. The numbers of $TUNEL^+$ or $BrdU^+$ β-cells were normalized to total islet area measured by a computer-assisted method using the Axion Vision 4 software (Zeiss, Goettingen, Germany).

Statistical analysis. All values are expressed as means ± SE of three independent experiments performed in triplicate to ensure reproducibility. Student's *t* test was used to compare values between two groups unless stated otherwise. Wilcoxon's rank-sum test was used when normal distribution of the data could not be ascertained. *P* values <0.05 were considered to represent statistically significant differences.

RESULTS

Remission of diabetes by imatinib. Initially, we studied the effect of imatinib on diabetes of *db/db* mice that develop classical obesity-induced diabetes. Intraperitoneal injection of 25 mg/kg imatinib to 5- to 7-week-old diabetic *db/db* mice (>16.7 mmol/l) daily (six times a week) for 1–4 weeks induced a dramatic reduction in blood glucose levels. Even after 1 week of treatment at 25 mg/kg, blood glucose levels, although still in the diabetic range, were significantly lower than those of control *db/db* mice treated with PBS ($P < 0.01$). The decrease of blood glucose levels was more prominent 2–4 weeks after imatinib treatment ($P < 0.005$), and blood glucose levels at 4 weeks after treatment were not significantly different from those of control C57BL/6 mice ($P > 0.1$) (Fig. 1*A*). We also treated *db/db* mice with imatinib at 50–75 mg/kg daily, which has been used for treatment of murine models of cancer (8,20). In this case, blood glucose levels were normalized since 1 week after commencing treatment, demonstrating more dramatic effects (data not shown). However, at these higher doses, imatinib produced adverse effects, i.e., peritoneal adhesion and generalized weakness. In contrast, imatinib at 25 mg/kg had no evident adverse effect after 4 weeks. Biopsies of the liver, muscle, kidney, heart, spleen, and other major organs conducted after 4 weeks of 25 mg/kg imatinib treatment showed no detectable abnormalities (supplementary Fig. 1, available in an online appendix at http://dx.doi.org/10.2337/db08-0080). Moreover, laboratory profile did not show any abnormality in blood cell counts or chemistry in *db/db* mice treated with 25 mg/kg imatinib for 4 weeks except hematocrit that was significantly decreased compared with control *db/db* mice but still within normal limits (supplementary Table 1, available in the online appendix). In addition, administration of 25 mg/kg imatinib for 4 weeks did not affect blood glucose levels, body weights, or food intake in control C57BL/6 mice ($P > 0.1$ for all points) (Fig. 1*A*–*C*), suggesting that imatinib at 25 mg/kg for 4 weeks has no significant toxicity or anorexic effect on mice. Based on these results, we used the daily 25 mg/kg protocol throughout the remainder of our study. Treatment with 25 mg/kg imatinib for 4 weeks induced a small but significant decrease in body weight ($P < 0.05 \sim 0.005$ at 2–4 weeks) (Fig. 1*B*) and food intake ($P < 0.01$) (Fig. 1*C*) of *db/db* mice. However, blood glucose levels in pair-fed *db/db* mice were not significantly different from those in control *db/db* mice fed ad libitum ($P > 0.1$ at all points) (Fig. 1*D*), suggesting that reduction in blood glucose level by imatinib cannot be explained by changes in food intake. Body weight of pair-fed *db/db* mice was slightly but significantly decreased compared with control *db/db* mice fed ad libitum ($P < 0.05$ at 4 week) but not significantly different from that of *db/db* mice treated with imatinib ($P > 0.1$ at all points) (Fig. 1*B*), indicating that pair feeding was done properly and reduction in blood glucose level by imatinib cannot be explained by changes in food intake or body weight.

Reduction of insulin resistance by imatinib. To assess glucose homeostasis and insulin sensitivity in *db/db* mice treated with imatinib, we conducted IPGTT after 25 mg/kg imatinib administration for 4 weeks. Blood glucose levels in control diabetic *db/db* mice after intraperitoneal injection of 1 g/kg glucose were significantly higher than those in control C57BL/6 mice, as expected ($P < 0.001$ for all points). In contrast, blood glucose levels after glucose challenge to *db/db* mice treated with 25 mg/kg imatinib for 4 weeks were significantly lower than those in control *db/db* mice ($P < 0.001$ for all points) and were similar to those of control C57BL/6 mice ($P > 0.1$ for all points) (Fig. 2*A*). To examine the effect of imatinib on insulin sensitivity, ITT was performed after 4 weeks of 25 mg/kg imatinib administration. Intraperitoneal injection of 1 unit/kg regular insulin to diabetic *db/db* mice did not decrease blood glucose level below untreated level, suggesting insulin resistance in those mice. In contrast, blood glucose levels in *db/db* mice treated with 25 mg/kg imatinib for 4 weeks were significantly lower than those in control *db/db* mice since 30 min after insulin injection ($P < 0.05$–0.005) and were not different from those in C57BL/6 mice ($P > 0.1$ for

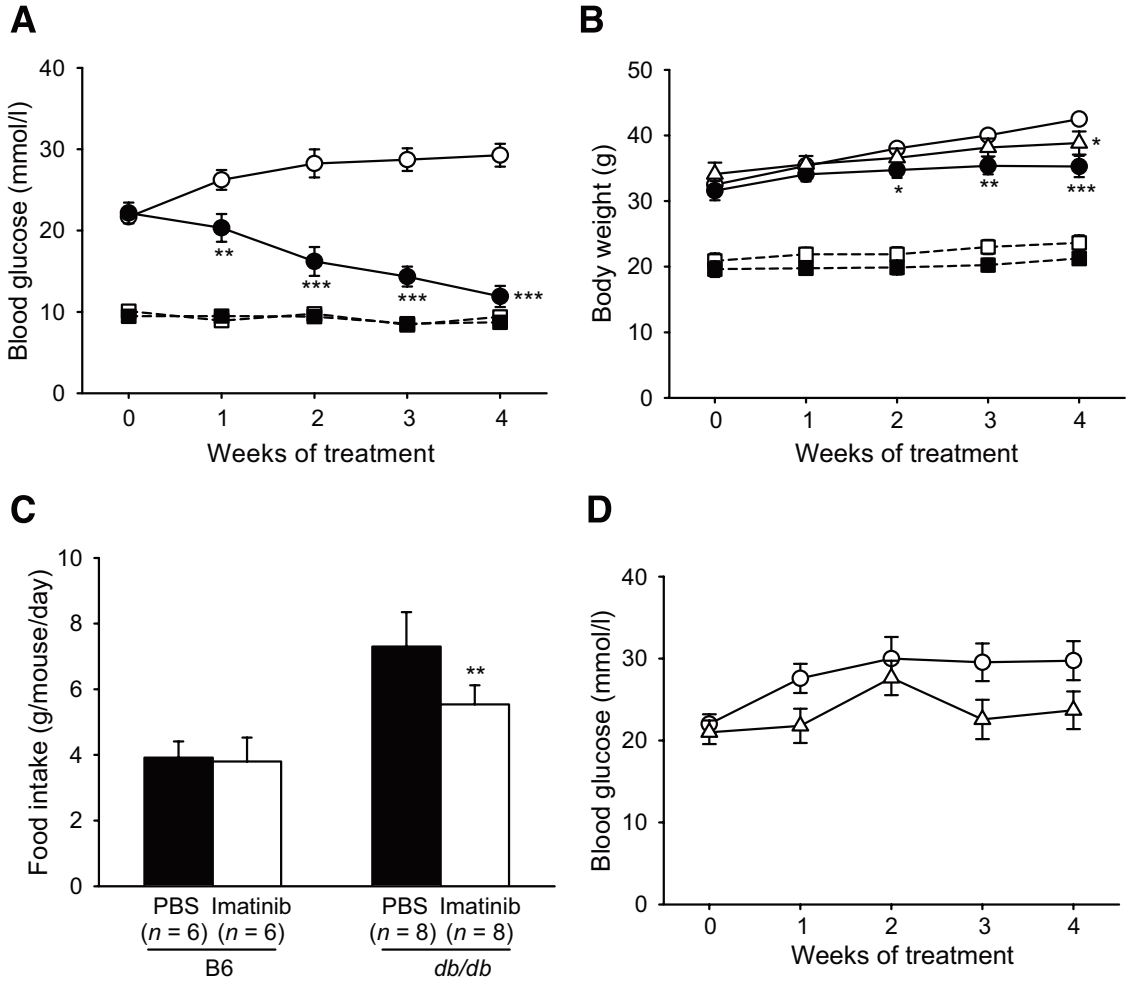

FIG. 1. Effect of imatinib on diabetes of *db/db* mice. *A*: Imatinib (25 mg · kg^{-1} · day^{-1}) was administered intraperitoneally to ~5- to 7-week-old diabetic *db/db* mice and control C57BL/6 mice for 4 weeks, and nonfasting blood glucose levels were determined. *B*: Body weight was monitored during treatment of *db/db* or C57BL/6 mice with 25 mg/kg imatinib or PBS. That of pair-fed *db/db* mice was also shown. *C*: Food intake of *db/db* and C57BL/6 mice treated with 25 mg/kg imatinib for 4 weeks. Food intake during the last week of the treatment was compared. *D*: Nonfasting blood glucose levels were determined in control *db/db* mice and pair-fed *db/db* mice. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$ vs. control *db/db* mice treated with PBS. ○, *db/db*-PBS (*A* and *B*, $n = 13$; *D*, $n = 7$); ●, *db/db*-Imatinib (*A* and *B*, $n = 13$); □, B6-PBS (*A* and *B*, $n = 8$); ■, B6-Imatinib (*A* and *B*, $n = 8$); △, *db/db*-Pair-fed (*B* and *D*, $n = 6$).

all points), suggesting that imatinib normalizes blood glucose levels in *db/db* mice by enhancing insulin sensitivity (Fig. 2*B*). Probably because of the decrease of insulin resistance, fasting serum insulin level (0 min) in control *db/db* mice that was markedly higher than that in C57BL/6 mice ($P < 0.001$) was significantly reduced by imatinib treatment for 4 weeks as determined by Wilcoxon's rank-sum test ($P < 0.05$) (Fig. 2*C*). To examine the changes of β-cell function by imatinib treatment, in vivo insulin response to intraperitoneal injection of 1 g/kg glucose was measured. Insulinogenic index calculated from serum insulin level before (0 min) and 15 min after glucose challenge was not significantly different between imatinib-treated and control *db/db* mice ($P > 0.1$) (Fig. 2*C*), suggesting that reduction of insulin resistance but not the improved insulin secretion explains most of the remission of diabetes in *db/db* mice by imatinib treatment.

Reduction of endoplasmic reticulum stress responses by imatinib in vivo. We next studied the mechanism underlying the improved insulin sensitivity in imatinib-treated *db/db* mice. We first studied the effect of imatinib on endoplasmic reticulum stress because endoplasmic reticulum stress has been reported to play a causative role in insulin resistance (6) and because Abl kinase, which is a target of imatinib, has been reported to play a role in the initiation of endoplasmic reticulum stress and the mediation of endoplasmic reticulum stress responses (21,22). When endoplasmic reticulum stress markers were examined in the liver and adipose tissues of fasted control *db/db* mice, the expression of phospho-PERK, phospho-eIF2α, TRB3, and CHOP was found to be markedly increased as previously reported (6). Intriguingly, upregulated expression of endoplasmic reticulum stress molecules was remarkably reduced both in the liver and adipose tissues by imatinib at 25 mg/kg for 4 weeks, suggesting that imatinib leads to the improvement of diabetes in *db/db* mice by attenuating endoplasmic reticulum stress (Fig. 3*A*).

Next, we examined JNK activation because JNK activation is an important component of endoplasmic reticulum stress responses (23) and is critically involved in obesity-induced insulin resistance (7,24). JNK was activated in the liver and adipose tissues of control *db/db* mice, and this was markedly reduced by imatinib treatment for 4 weeks (Fig. 3*B*), consistent with the reductions in the expression of other endoplasmic reticulum stress molecules, such as phospho-PERK and CHOP, and also with previous papers

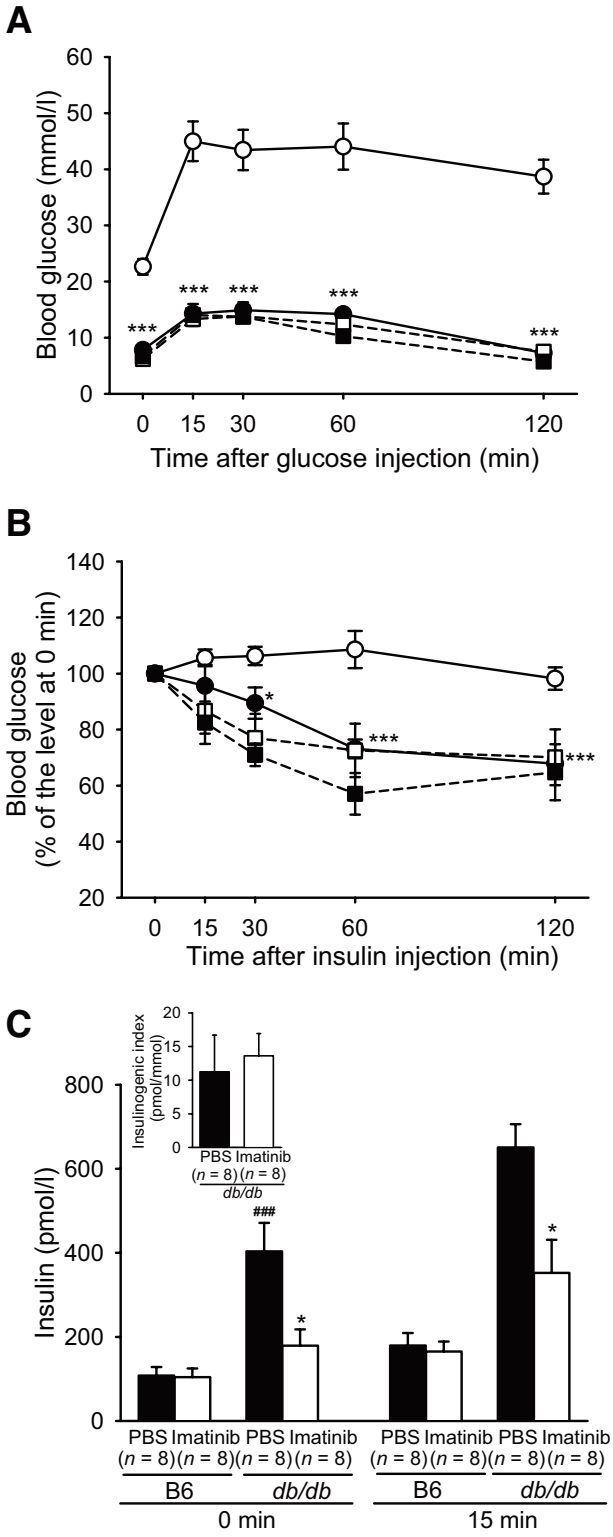

FIG. 2. Effect of imatinib on insulin resistance of *db/db* mice. *A*: IPGTT was conducted by injecting 1 g/kg glucose intraperitoneally into *db/db* mice or control C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks and measuring blood glucose levels at the indicated time points. *B*: ITT was done by injecting 1 unit/kg regular insulin intraperitoneally into *db/db* mice or control C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks and measuring blood glucose levels at the indicated time points. *C*: Serum was obtained from *db/db* mice or control C57BL/6 mice treated with 25 mg/kg imatinib or PBS before (0 min) and 15 min after intraperitoneal injection of 1 g/kg glucose, and insulin levels were measured by enzyme-linked immunosorbent assay. Insulinogenic index was calculated using insulin levels at 0 and 15 min. *$P < 0.05$; ***$P < 0.005$ vs. control *db/db* mice treated with PBS (15);

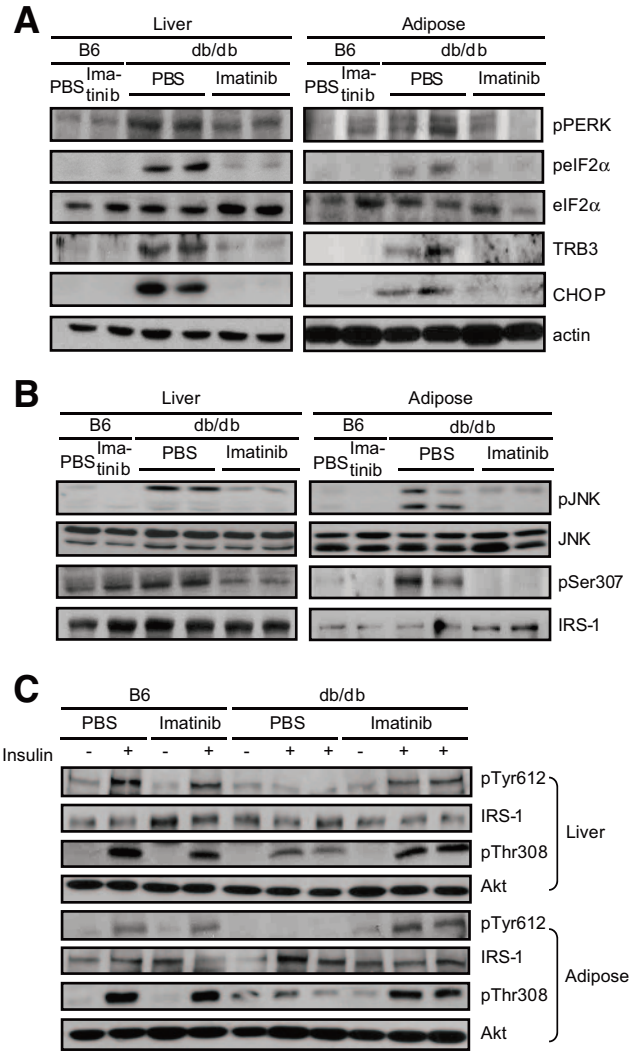

FIG. 3. Attenuation of endoplasmic reticulum stress in the insulin target tissues by imatinib treatment. *A*: Expression of endoplasmic reticulum stress markers, such as phospho-PERK, phospho-eIF2α, TRB, and CHOP, were determined by Western blotting of the liver and adipose tissues from fasted *db/db* and C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks. *B*: Insulin resistance markers in the liver and adipose tissues of *db/db* and C57BL/6 mice were studied by Western blotting using antibody to phospho-JNK or phospho-IRS-1 Ser307. *C*: In vivo insulin signaling was examined by investigating phosphorylation of IRS-1 Tyr612 and Akt Thr308 using Western blotting of the liver and adipose tissues from imatinib-treated *db/db* mice, PBS-treated *db/db* mice, and C57BL/6 mice 5 min after insulin injection into the tail vein.

showing an important role for c-Abl kinase in JNK activation (25,26). We next investigated whether insulin signaling is improved by imatinib because of amelioration of endoplasmic reticulum stress. We studied the serine phosphorylation of IRS-1 because Ser307 phosphorylation of IRS-1 by activated JNK plays an important role in insulin resistance by inhibiting subsequent insulin signaling through IRS-1 (27). IRS-1 Ser307 phosphorylation was increased in the liver and adipose tissues of *db/db* mice compared with C57BL/6 mice, as expected, and imatinib treatment for 4 weeks attenuated the increased IRS-1 Ser307 phosphorylation (Fig. 3*B*). When we examined insulin signaling after 5 units/kg insulin injection in vivo,

###$P < 0.005$ vs. control C57BL/6 mice treated with PBS. ○, *db/db*-PBS (*A*, $n = 10$; *B*, $n = 8$); ●, *db/db*-Imatinib (*A*, $n = 10$; *B*, $n = 8$); □, B6-PBS (*A* and *B*, $n = 8$); ■, B6-Imatinib (*A* and *B*, $n = 8$).

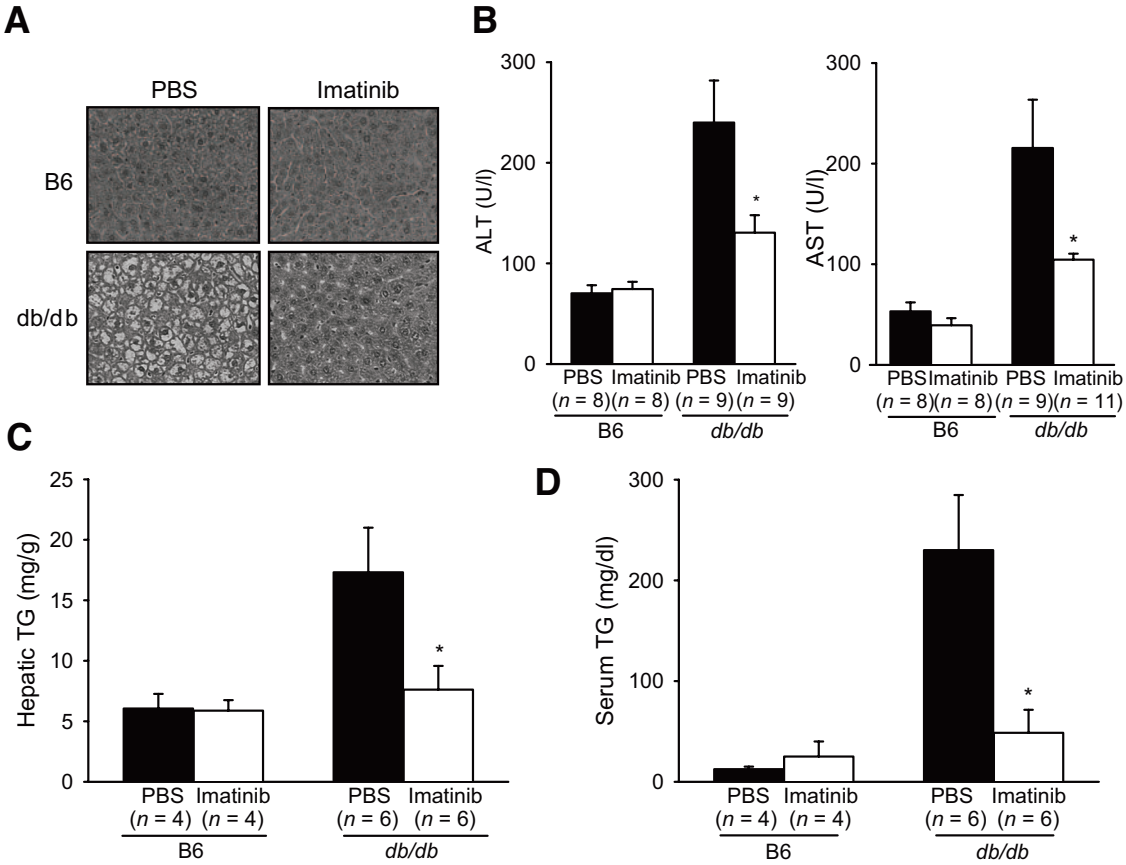

FIG. 4. Amelioration of steatosis of *db/db* mice by imatinib. *A*: Liver sections were prepared from *db/db* or control C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks, and hematoxylin-eosin staining was done. *B*: Serum was obtained from fasted *db/db* or C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks, and ALT/AST levels were measured using a chemical analyzer. *C*: Lipid was extracted from the liver of *db/db* or C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks using chloroform/ethanol mixture, and tissue TG levels were measured using Free Glycerol Reagent containing lipase. *D*: Serum was obtained from fasted *db/db* or C57BL/6 mice treated with 25 mg/kg imatinib or PBS for 4 weeks, and serum TG levels were measured using a blood chemistry analyzer. *$P < 0.05$ vs. control *db/db* mice treated with PBS.

phosphorylation of IRS-1 at Tyr612 (28) and that of Akt at Thr308 downstream of IRS-1 (29) were reduced in the liver and adipose tissues of *db/db* mice compared with C57BL/6 mice, as expected (Fig. 3*C*). Here, imatinib treatment for 4 weeks restored the reduced IRS-1 Tyr612 and Akt Thr308 phosphorylation in response to insulin (Fig. 3*C*), suggesting that imatinib improved insulin signaling through IRS-1 and Akt by decreasing JNK activation and IRS-1 Ser307 phosphorylation.

We next studied changes of the lipid metabolism in the liver because obesity-induced insulin resistance is associated with fatty liver disease both in human and mice. Microscopic examination revealed a remarkable reduction in degree of steatosis in *db/db* mice treated with imatinib at 25 mg/kg for 4 weeks (Fig. 4*A*). Consistent with this improvement of steatosis, serum ALT/AST levels and hepatic TG contents were significantly lower in imatinib-treated *db/db* mice compared with control *db/db* mice as determined by Wilcoxon's rank-sum test ($P < 0.05$) (Fig. 4*B* and *C*). The same test also showed that serum TG was significantly lower in imatinib-treated *db/db* mice compared with control *db/db* mice ($P < 0.05$) (Fig. 4*D*).

We next studied pancreatic β-cell changes because pancreatic β-cells have adaptive capacity to changing insulin resistance and β-cell failure is the ultimate cause of diabetes (30,31). Relative β-cell area in *db/db* mice was significantly increased by imatinib treatment for 4 weeks ($P < 0.05$) (Fig. 5*A*). To study the mechanism underlying this effect, we conducted TUNEL staining combined with insulin immunohistochemistry. Numbers of TUNEL$^+$ apoptotic β-cells per unit islet area were significantly decreased by imatinib treatment ($P < 0.05$) (Fig. 5*B*). We also counted the numbers of BrdU-stained β-cells as a measure of β-cell proliferation. Numbers of BrdU$^+$-proliferating β-cells per unit islet area were significantly increased by imatinib treatment ($P < 0.05$) (Fig. 5*C*), suggesting that imatinib protects β-cell mass by reducing β-cell death and increasing β-cell proliferation after reduction of insulin resistance.

Reduction of endoplasmic reticulum stress responses by imatinib in vitro. Next, we studied whether the observed amelioration of endoplasmic reticulum stress in vivo by imatinib is due to the direct cell-autonomous effect of imatinib on endoplasmic reticulum stress or an indirect result of a complex in vivo interaction among diverse pathways. When endoplasmic reticulum stress was induced in HepG2 hepatoma cells by 250–1,000 nmol/l thapsigargin or 0.1–1 μg/ml tunicamycin (32), expression of phospho-PERK, phospho-eIF2α, and phospho-JNK was increased, as expected. Here, combined treatment with 10 μmol/l imatinib markedly downregulated the expression of all three of these endoplasmic reticulum stress molecules in HepG2 cells, suggesting that imatinib directly ameliorates cellular endoplasmic reticulum stress (Fig. 6*A* and *B*). In contrast, the same imatinib treatment did not affect JNK activation by anisomycin that is known to

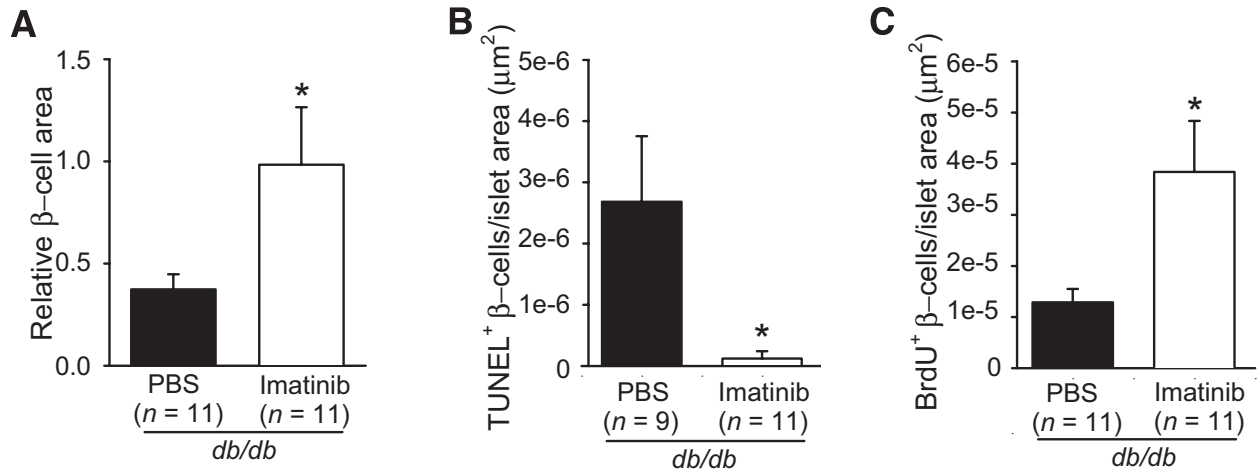

FIG. 5. Changes in β-cell mass by imatinib treatment. *A*: After immunostaining of pancreatic sections from imatinib-treated or control *db/db* mice using anti-insulin antibody as the primary antibody, point-counting morphometry was conducted to measure the relative β-cell area. *B*: After TUNEL staining of pancreatic sections from imatinib-treated or control *db/db* mice, immunohistochemistry using anti-insulin antibody was performed. Numbers of TUNEL$^+$ apoptotic β-cells stained with anti-insulin antibody were counted and normalized to islet area measured by a computer-assisted method. *C*: Immunostaining using anti-BrdU antibody was conducted on pancreatic sections from of imatinib-treated or control *db/db* mice to which 100 μg/g BrdU was injected intraperitoneally. After subsequent immunohistochemistry using anti-insulin antibody, numbers of BrdU$^+$ β-cells were calculated and normalized as in *B*. *$P < 0.05$ vs. control *db/db* mice treated with PBS.

activate JNK independent of endoplasmic reticulum stress (6), thus demonstrating the specificity of the effect of imatinib on endoplasmic reticulum stress–related JNK activation (Fig. 6*C*).

DISCUSSION

Observations made in patients with CML and concomitant type 2 diabetes suggest that imatinib might have a beneficial effect on diabetes (10,11). However, the observed reductions in blood glucose levels in patients with CML and type 2 diabetes might have been caused by nonspecific effects attributable to improved general health status achieved by treating CML with imatinib, and thus, those reports do not allow an assessment of the effect of imatinib on diabetes itself. Furthermore, one study concluded that imatinib has no effect on human type 2 diabetes (33). While this work was in preparation, a study showing the effect of imatinib on diet-induced obesity and insulin resistance was published (34). However, the mechanism of the amelioration of the insulin resistance by imatinib was not investigated, and the effect of imatinib on diabetic *db/db* mice with elevated blood glucose levels was not tested. In fact, imatinib administration increased blood glucose levels in rats on high-fat diet (34). The present study clearly demonstrates that imatinib dramatically reduces elevated blood glucose levels and has therapeutic effects on type 2 diabetes, at least in this animal model.

In addition to the remission of diabetes, imatinib administration induced a small but significant decrease of body weight and food intake in *db/db* mice. Such effects might be due to the improved anorexigenic effect of insulin caused by increased insulin sensitivity after imatinib administration (35). Possible direct effect of imatinib on the hypothalamus or systemic toxic effect of imatinib cannot be eliminated as potential causes of the decreased body weight. However, we consider that the possibility of systemic toxicity is quite low because the dose of imatinib used in this investigation was much lower than that used in animal models of cancer (8,20). Moreover, imatinib administration did not affect the body weight or food intake of C57BL/6 mice. Histological analysis of the major organs and blood cell/chemistry profiles produced no evidence of systemic toxicity, further supporting the absence of significant systemic toxicity.

Imatinib has been reported to affect glucose metabolism, e.g., it reduces glucose utilization by cancer cells (36,37). In fact, this has been considered to be a mechanism of tumor regression by imatinib (37). However, reduced glucose utilization cannot explain the improved glucose control in patients or animals with type 2 diabetes. In the present study, we explored the possibility that endoplasmic reticulum stress modulation by imatinib induces the remission of diabetes because c-Abl or Bcr-Abl kinase has been reported to participate in cellular response to endoplasmic reticulum stress (21) or in the initiation of endoplasmic reticulum stress by reducing endoplasmic reticulum Ca^{2+} content (22). Consistent with our hypothesis, expression of endoplasmic reticulum stress molecules in insulin target tissues of *db/db* mice was markedly reduced by imatinib treatment. These effects of c-Abl kinase and its inhibitor imatinib on endoplasmic reticulum stress responses are consistent with the abrogation of endoplasmic reticulum stress–induced cell death or JNK activation in *c-Abl*–null cells (21,25) and appear to be related to the localization of c-Abl kinase in endoplasmic reticulum and mitochondria as well as cytoplasm and nucleus (21,22). Because endoplasmic reticulum stress has been reported to play an important role in obesity-induced insulin resistance (6) and because chemical chaperones relieving endoplasmic reticulum stress have been shown to reverse diabetes (38), our findings provide a rational explanation for the effect of imatinib on diabetes in *db/db* mice. A recent article suggested that activation of endoplasmic reticulum stress after imatinib is a cause of imatinib-induced cardiomyopathy (39). However, the dose of imatinib used in that study (50–200 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3–6 weeks) was much higher than that used in this investigation, and the response to imatinib may be dose and tissue dependent.

Increase of β-cell mass by imatinib treatment could be also attributable to the reduction of endoplasmic reticulum stress in β-cells that has been observed in obese subjects or animals with type 2 diabetes (30,31), although we did not explore this possibility further and the ultimate

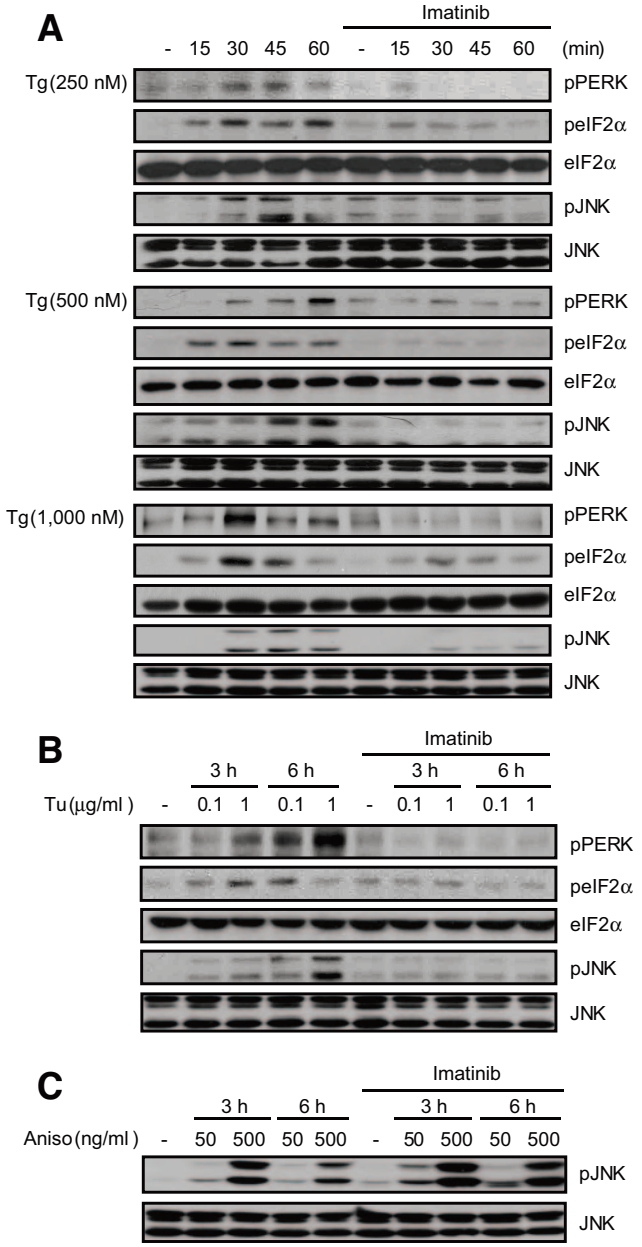

FIG. 6. Effect of imatinib on endoplasmic reticulum stress in vitro. HepG2 hepatoma cells were treated with 250–1,000 nmol/l thapsigargin (Tg) (*A*), 0.1–1 μg/ml tunicamycin (Tu) (*B*), or anisomycin (Aniso) (*C*) with or without 10 μmol/l imatinib pretreatment for 1 h. Western blotting was conducted using cell lysate and the indicated antibody as the primary antibody.

causal relationship between the improved glycemic control and improved β-cell mass remains to be clarified.

The molecular and cellular mechanisms of endoplasmic reticulum stress attenuation by imatinib are unknown. Changes in endoplasmic reticulum Ca^{2+} content by c-Abl or imatinib (22) may be involved in this modulation of endoplasmic reticulum stress by imatinib. We observed the cell-intrinsic effects of imatinib on endoplasmic reticulum stress induced by thapsigargin and tunicamycin that are typical endoplasmic reticulum stressor agents inhibiting endoplasmic reticulum Ca^{2+}-ATPase and posttranslational protein glycosylation at endoplasmic reticulum, respectively (40). However, other indirect effects of imatinib on endoplasmic reticulum stress may also play a role in the reduction of endoplasmic reticulum stress in vivo. For instance, imatinib has been reported to inhibit TNF-α production in the liver (41) that may be related to endoplasmic reticulum stress observed in obesity-induced insulin resistance (42).

The decreased JNK activation by imatinib observed in the liver of *db/db* mice is consistent with amelioration of endoplasmic reticulum stress, reduced IRS-1 Ser307 phosphorylation, and an increase in insulin-induced phosphorylation of IRS-1 Tyr612 or Akt Thr308, because JNK activation is an important part in endoplasmic reticulum stress response (23) and because JNK activation has been reported to induce insulin resistance by phosphorylating IRS-1 at Ser307 (27). These results are also consistent with previous reports showing reduced JNK activation in leukemic cells by imatinib (26) or those reporting a critical role for c-Abl kinase in JNK activation by anticancer agents or growth factors (25,43). However, these findings are at odds with those of another study reporting decreased constitutive IRS-1 signaling after imatinib treatment of leukemic cells (44). The effect of imatinib on constitutive IRS-1 signaling in cancer cells might differ from that on insulin-induced IRS-1 signaling in insulin target tissues.

The above findings suggest the possibility that the target of imatinib involved in the improvement of diabetes in *db/db* mice is c-Abl kinase itself. This speculation is supported by a previous observation that downregulation of c-Abl mRNA by siRNA protected insulinoma cells from cytokine-induced death (13). Imatinib was originally developed as a specific inhibitor of Abl kinase, however, other targets have been identified, such as Kit and platelet-derived growth factor receptor kinases (9,45). Hence, the existence of other molecular species that mediate the antidiabetic effect of imatinib cannot be eliminated. Because imatinib was initially developed for life-threatening diseases and has common side effects that may prevent its development as an antidiabetic agent, it is unclear whether imatinib or its related compounds could be used as therapeutic agents against diabetes. However, if more specific inhibitors of Abl kinase could be developed or new molecular targets of imatinib mediating its antidiabetic effects could be identified, they would provide excellent bases for the development of new antidiabetic agents.

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