Inhibition of Dipeptidyl Peptidase IV With Sitagliptin (MK0431) Prolongs Islet Graft Survival in Streptozotocin-Induced Diabetic Mice

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OBJECTIVE—Dipeptidyl peptidase-IV (DPP-IV) inhibitors have been introduced as therapeutics for type 2 diabetes. They partially act by blocking degradation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), thus increasing circulating levels of active hormones. In addition to their insulinotropic actions, GLP-1 and GIP also promote β-cell proliferation and survival, and DPP-IV inhibitors exert similar effects in rodent type 2 diabetes models. The study objective was to establish whether DPP-IV inhibitor treatment prolonged survival of transplanted islets and to determine whether positron emission tomography (PET) was appropriate for quantifying the effect of inhibition on islet mass.

RESEARCH DESIGN & METHODS—Effects of the DPP-IV inhibitor MK0431 (sitagliptin) on glycemic control and functional islet mass in a streptozotocin (STZ)-induced type 1 diabetes mouse model were determined with metabolic studies and microPET imaging.

RESULTS—The type 1 diabetes mouse model exhibited elevated plasma DPP-IV levels that were substantially inhibited in mice on an MK0431 diet. Residual β-cell mass was extremely low in STZ-induced diabetic mice, and although active GLP-1 levels were increased by the MK0431 diet, there were no significant effects on glycemic control. After islet transplantation, mice fed normal diet rapidly lost their ability to regulate blood glucose, reflecting the suboptimal islet transplant. By contrast, the MK0431 group fully regulated blood glucose throughout the study, and PET imaging demonstrated a profound protective effect of MK0431 on islet graft size.

CONCLUSIONS—Treatment with a DPP-IV inhibitor can prolong islet graft retention in an animal model of type 1 diabetes. ***Diabetes*** **57:1331–1339, 2008**

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AMC, aminomethylcoumarin; AUC, area under the curve; DPP-IV, dipeptidyl peptidase-IV; [^{18}F]FHBG, 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)-guanine; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; IPGTT, intraperitoneal glucose tolerance test; m.o.i., multiplicity of infection; PET, positron emission tomography; rAD-TK, recombinant adenovirus expressing *HSV1-Sr39TK*; ROI, region of interest; STZ, streptozotocin.

Over the past few years, significant progress has been made in the development of protocols for transplantation of human pancreatic islets in type 1 diabetes (1). However, in recent studies only 67% (2) and 10% (3) of transplant recipients were insulin independent at the end of 1 and 5 years, respectively. Additionally, at least two donor pancreata are generally needed per transplantation (4) because of the loss of viable islets both during isolation and after transplantation. The causes of posttransplant islet loss are multiple and probably include deficiencies in survival factors (5); altered islet vasculature resulting in deficient nutrient and oxygen delivery (6,7), inflammation, and immune-mediated destruction; and the toxic effects of immunosuppressive agents (7). There is therefore considerable interest in developing methods for improving the viability of isolated islets and prolonging their survival.

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gastrointestinal hormones that potentiate glucose-stimulated insulin secretion, i.e., they are classified as incretins. Additionally, they both stimulate insulin biosynthesis and stimulate proliferation of the β-cell while inhibiting apoptosis (8–12). Termination of the actions of both GIP and GLP-1 is performed by dipeptidyl peptidase-IV (DPP-IV; CD26) (13–15), an integral membrane glycoprotein that is also present in soluble form in blood plasma. DPP-IV acts on oligopeptides by selectively removing NH_2-terminal dipeptides (16,17). Although GIP and GLP-1 exert powerful prosurvival actions on the islet, their short half-lives in the circulation limit their potential usefulness as therapeutic agents. Members of two classes of compounds that circumvent this problem have recently been approved by the U.S. Food and Drug Administration: the DPP-IV–resistant GLP-1 receptor agonist (incretin mimetic) exenatide (Byetta) and the DPP-IV inhibitor sitagliptin (Januvia). Both have been shown to profoundly improve glucose homeostasis in type 2 diabetes (17–19).

There have been relatively few studies on the potential for incretin mimetics or DPP-IV inhibitors in the treatment of type 1 diabetes. Infusion or subcutaneous GLP-1 lowered fasting hyperglycemia and reduced glycemic excursions and requirements for exogenous insulin in type 1 diabetic patients (20,21). In preclinical studies, the DPP-IV inhibitor isoleucine thiazolidide improved glucose tolerance in both streptozotocin (STZ)-induced (22,23) and BioBreeding (BB) (23) diabetic rats, with clear islet protection in the former group (22). The latter observation suggested that treatment with a DPP-IV inhibitor could also protect transplanted islets by increasing the endogenous levels of incretin hormones. In the current study, we

examined the effect of administering the DPP-IV inhibitor sitagliptin on survival of islets transplanted under the kidney capsule of STZ-induced diabetic mice. Islet survival was assessed from fasting plasma glucose, intraperitoneal glucose tolerance tests (IPGTTs), and positron emission tomography (PET) imaging.

RESEARCH DESIGN AND METHODS

Male C57BL/6 mice (10–12 weeks old) were obtained from the UBC (University of British Columbia) Animal Care Facility and the Jackson Laboratory (Bar Harbor, ME). After 3–5 days' acclimation, the animals received a single dose of STZ (200 mg/kg). At 1 week after STZ administration, the mice were placed on either a normal chow diet (rodent chow no. 5015; Purina) or diet containing sitagliptin (rodent chow no. 5015 [Purina] plus 4 g/kg MK0431 [Research Diets, New Brunswick, NJ]) (24) ad libitum. All animal experiments were conducted in accordance with the guidelines put forth by the University of British Columbia committee on animal care and the Canadian Council on Animal Care.

DPP-IV activity assays. To measure the plasma DPP-IV activity, a Gly-Pro-aminomethylcoumarin (AMC) fluorometric assay was used. Gly-Pro-AMC is cleaved by the enzyme to release the fluorescent AMC. A typical reaction contained purified DPP-IV enzyme for standard reaction or plasma sample, 50 μmol/l Gly-Pro-AMC, and 100 mmol/l Tris buffer, pH 8.0. Liberation of AMC was monitored using an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Measurement of total pancreatic insulin content. Pancreata were homogenized in 5 ml of ice-cold 2 mol/l acetic acid, boiled, and centrifuged (10 min, 15,000 rpm, 4°C). The insulin concentration in neutralized supernatants was determined by radioimmunoassay (Linco Research) and normalized for protein concentration (bicinchoninic acid; Pierce, Rockford, IL).

Islet isolation and cell culture. Male C57BL/6 mice (10–12 weeks old) were anesthetized by intraperitoneal injection of pentobarbital (30–40 mg/kg) and islets isolated by collagenase digestion, followed by filtration through a 70-μm nylon mesh cell strainer (25). Islets were hand-picked and cultured for 1–2 h before adenoviral infection in RMPI 1640 (Sigma) supplemented with 5.5 mmol/l glucose, 0.25% HEPES, 10% fetal bovine serum, 100 units/ml penicillin G-sodium, and 100 μg/ml streptomycin sulfate (Invitrogen).

Generation of recombinant adenoviruses and islet gene transfer. Recombinant adenovirus expressing *HSV1-Sr39TK* was produced and expanded by infection of HEK-293 (human embryonic kidney) cells, and multiplicity of infection (m.o.i.) was determined by plaque assays (26). Islets were exposed to 250 m.o.i. of purified recombinant adenoviruses for 2 h at 37°C and washed three times with Hanks' balanced salt solution. Islets were then recultured for 16–24 h in RPMI 1640 medium (Sigma) supplemented with 5.5 mmol/l glucose, 0.25% HEPES, 10% fetal bovine serum, 100 units/ml penicillin G-sodium, and 100 μg/ml streptomycin sulfate (Invitrogen) until transplanted or assayed for in vitro studies.

Islet transplantation. STZ (200 mg/kg) was injected intraperitoneally into C57BL/6 mice (male, 10–12 weeks old) 5 days before transplantation, and the mice with blood glucose levels ≥20 mmol/l for 3 consecutive days were considered as diabetic. Under general anesthesia (induced by isoflurane inhalation), a lombotomy was performed, and the kidneys were exposed. A breach was made in the kidney capsule, and a polyethylene catheter was introduced through the breach and advanced in the subcapsular space to the opposite pole of the kidney. For all experiments 300 islets (not islet equivalents), obtained from C57BL/6 mice and infected with 250 m.o.i. of recombinant adenovirus expressing *HSV1-Sr39TK* (rAD-TK), were slowly injected and allowed to spread at the pole. The catheter was then retrieved, the opening cauterized, and the kidney repositioned, followed by suturing of muscle and skin. Mice were allowed to regain consciousness and placed on either a normal chow diet (Purina rodent chow no. 5015) or diet containing sitagliptin (Purina rodent chow no. 5015 plus 4 g/kg MK0431) ad libitum (24).

Plasma glucose determinations, IPGTTs, and plasma hormone measurements. Nonfasting blood glucose levels were measured in mouse tail blood using a SureStep glucose analyzer (LifeScan) at the time points indicated in Figs. 2, 3, and 4. For the IPGTTs, mice were fasted for 4 h and blood glucose levels measured at 0, 15, 30, 60, 90, and 120 min after the glucose challenge (2 g/kg). Blood samples with glucose levels ≥27.8 mmol/l were diluted with blood from nondiabetic mice and levels calculated. Plasma insulin, glucagon, and active GLP-1 levels were determined using a Multiplex assay kit (Linco Research).

Synthesis of 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)-guanine. 9-(4-[^{18}F]-Fluoro-3-hydroxymethylbutyl)-guanine ([^{18}F]FHBG) was synthesized as previously described (27).

MicroPET scanning. Transplanted mice were scanned (27,28) on day 1 (1st week) and days 8 (2nd week), 15 (3rd week), and 22 (4th week) after the transplantation using a Focus120 (CTI Concorde) system that yield 95 slices, 1.2 mm apart with an in-plane resolution of 1.4 mm FWHM (full-width at half-maximum) (29). The mice were transported to the PET suite in their home cage. Shortly before scanning, they were anesthetized by isoflurane inhalation and placed on the scanner bed in a prone position, and a nose cone was fitted over the face to maintain isoflurane anesthesia throughout the procedure. An intravenous catheter (27 G) was placed in the tail vein, and saline was slowly dripped to maintain catheter patency. [^{18}F]FHBG (100 μCi in 0.5 ml) was injected as a bolus in the tail vein. Scanning in list mode started on tracer injection and lasted for 1 h.

Image reconstruction and data analysis. The data were subsequently framed and reconstructed with filtered back projection. In addition, to improve organ identification and the accuracy of positioning regions of interest (ROIs), a single time frame from 30–60 min postinjection was reconstructed using the three-dimensional iterative maximum a posteriori algorithm (30), which yields images of higher resolution (31). Activity in the kidney area was usually visualized in 3–4 slices. To reduce the influence of partial volume effects, the same pixel sizes of circular ROIs were drawn on the target areas on the two best slices of the maximum a posteriori image containing the kidneys. The ROIs were then repositioned on the kinetically reconstructed data, and time activity curves were obtained for each region. The counts per pixel per minute obtained from the ROI were converted to counts per milliliter per minute by using a calibration constant obtained from scanning a cylinder phantom in the microPET scanner. The ROI counts per milliliter per minute were converted to counts per gram per minute, assuming a tissue density of 1 g/ml, and divided by the injected dose to obtain an image ROI-derived percentage injected dose of [^{18}F]FHBG retained in kidney.

Statistical analysis. Data are expressed as the means ± SE, with the number of individual experiments presented in the figure legends. Data were analyzed using the linear regression analysis program PRISM (GraphPad, San Diego, CA), and areas under the curve (AUCs) were calculated using the algorithm provided in the Prism software package. Significance was tested using ANOVA with the Newman-Keuls post hoc test ($P < 0.05$) as indicated in the figure legends.

RESULTS

Effects of orally administered MK0431 in STZ-induced diabetic mice. In previous studies it was shown that DPP-IV inhibitor treatment reduced the progressive severity of diabetes induced by STZ in the rat (22). In the single, high-dose STZ–induced diabetic mouse model used for the islet transplant studies, a higher level of β-cell ablation was expected. However, to establish that significant islet protection or growth would not occur, MK0431 was administered in the chow (4 g/kg) 1 week after STZ administration and the progression of diabetes studied. Plasma DPP-IV activity increased within the 1st week after STZ administration. However, activity was greatly inhibited in the MK0431-administered mice, whereas plasma DPP-IV activity in mice receiving the standard diet continued to rise to levels that were ~250% greater (Fig. 1*A*). Plasma levels of active GLP-1 were significantly increased in the diabetic mice treated with MK0431, demonstrating protection of circulating incretins from degradation (Fig. 1*B*).

As expected, food intake was enhanced in the STZ-induced diabetic mice, but in animals switched to chow containing MK0431, food intake decreased to levels similar to those before STZ administration (Supplementary Fig. 1*A*, which can be found in an online appendix, available at http://dx.doi.org/10.2337/db07-1639). Despite the lower food intake with the MK0431 diet, there were no significant differences in body weight between the two groups throughout the study, with an initial decrease and little change after the 1st week (Supplementary Fig. 1*B*). Water intake increased to similar levels in both groups (Supplementary Fig. 1*C*). MK0431 treatment had no significant effect on the highly elevated nonfasting blood glucose levels throughout (Fig. 2*A*). At the end of the 5-week test period, mean fasting glucose, IPGTT blood glucose re-

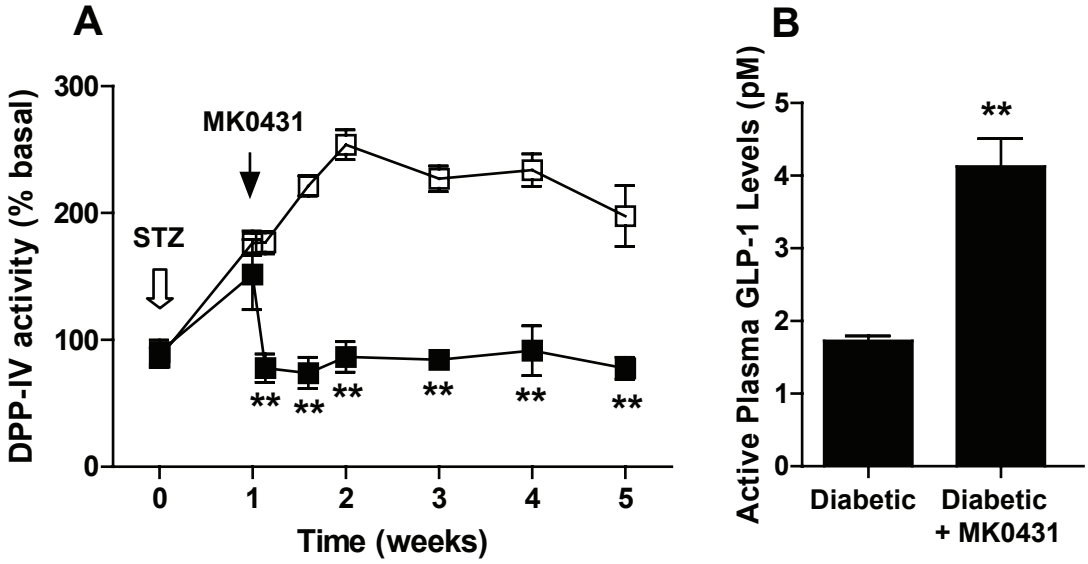

FIG. 1. MK0431 inhibits plasma DPP-IV activity and increases plasma active GLP-1 levels in STZ-induced diabetic mice. C57BL/6 mice administered with a single high dose of STZ (200 mg/kg) were placed on either a normal chow diet or diet containing MK0431 (4 g/kg). *A* and *B*: Plasma DPP-IV activity (*A*) and plasma active GLP-1 levels (*B*) 1 month after initiation of the MK0431 treatment. All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test. **$P < 0.05$ vs. diabetic, $n = 6$ mice per group. □, diabetic control; ■, diabetic control with MK0431 diet.

sponses (Fig. 2*B–D*), total pancreatic insulin content (Fig. 2*E*), and plasma insulin-to-glucagon ratios (Fig. 2*F*) did not differ between the MK0431 and normal chow diet–fed mice. Very few β-cells could be identified in histological sections of the pancreata (data not shown). MK0431 treatment also resulted in no significant changes in fasting or nonfasting blood glucose levels or IPGTT blood glucose responses in nondiabetic control mice (Supplementary Fig. 2*A–D*).

MK0431 treatment improves islet graft survival in STZ-induced diabetic mice. The effect of MK0431 on islet graft survival was next studied. Before transplantation under the kidney capsule, islets were infected with rAD-TK, which expresses *HSV1-Sr39TK* (the gene for a mutant form of herpes simplex virus 1 thymidine kinase), to allow PET imaging. It was previously determined that 250 m.o.i. was an appropriate dose for the administration with rAD-TK, based on the level of Sr39TK expression, insulin secretory capacity, and islet cell viability (28). At 48 h after infection with 250 m.o.i. rAD-TK, 27 ± 5% ($n = 3$) of β-cells were positively stained for thymidine kinase (Supplementary Fig. 3). The number of islets used for transplantation (300) was chosen so as to partially correct the hyperglycemia in STZ-administered C57BL/6 mice for a limited period (32). For the first 1.5 weeks after transplantation, both groups had similar body weights (Fig. 3*A*) and water intake (Fig. 3*B*). At that point, the MK0431 diet group began to gain body weight, whereas the normal chow diet group did not, and water intake in the latter group increased profoundly. At 3 days after transplantation, nonfasting blood glucose levels were normalized in the MK0431 diet group, whereas levels in the nontreated mice progressively increased from 1.5 weeks on (Fig. 3*C*). A similar trend was evident for fasting blood glucose (Fig. 3*D*). During the course of the study, ~40% of the mice placed on normal chow diet died, probably as a result of severe hyperglycemia, whereas all of the mice on the MK0431 diet survived (Fig. 3*E*). After islet transplantation food intake decreased. This was followed by a progressive rise in food consumption in the normal chow diet group to presurgery levels (Fig. 3*F*). However, food intake in the MK0431 group was significantly lower than the normal chow diet group from week 2 onward (Fig. 3*F*). Plasma DPP-IV activity was reduced by 78–88% in the MK0431 group (Fig. 3*G*) and plasma active GLP-1 levels were increased ~2.5-fold compared with the normal chow diet group (Fig. 3*H*). Though not reaching statistical significance, DPP-IV activity in the normal chow diet group showed a downward trend from 1 week after transplantation (Fig. 3*G*). There was no significant correlation between plasma DPP-IV activity and body weight, food consumption, water intake, or blood glucose levels.

IPGTTs were performed on both groups of mice at the 1st, 2nd, 3rd, and 4th week after transplantation ($n = 5$ animals/group). As expected, IPGTTs showed that transplanted islets in the normal chow diet group lost their capacity to regulate blood glucose levels over time because of the suboptimal dose of islets. By contrast, the MK0431 group preserved their capacity to regulate blood glucose levels normally until the end of the study (Fig. 4). Basal plasma insulin in the normal chow diet group was lower than in the MK0431-treated mice. Glucose-stimulated insulin secretion (GSIS) decreased greatly in the normal chow diet group over the 4 weeks after transplantation, whereas the MK0431 group showed stable insulin secretory responses to glucose stimulation throughout (Fig. 5*A*). Although plasma glucagon levels increased substantially in the normal chow diet group over the 3 weeks after transplantation, glucagon in the MK0431 group remained low (Fig. 5*B*). As a consequence, plasma insulin-to-glucagon ratios were drastically decreased in the normal chow diet group, whereas the MK0431 group showed constant ratios throughout, reflecting the control of blood glucose levels (Fig. 5*C*). Together, these results strongly suggest that MK0431 had positive effects on the regulation of hyperglycemia, potentially through prolongation of islet graft survival.

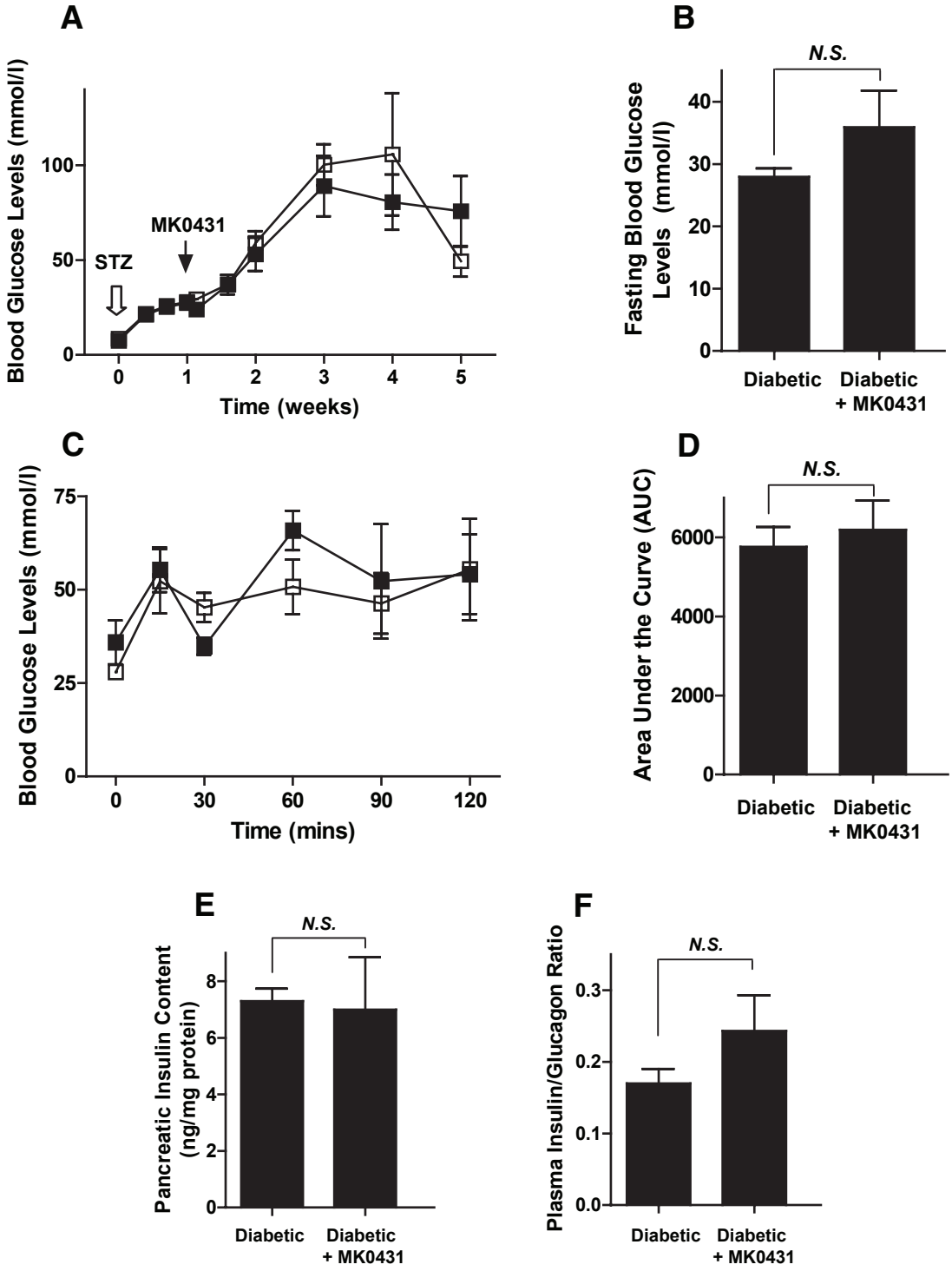

FIG. 2. The effects of MK0431 on glycemic control in STZ-induced diabetic mice. *A–F*: Nonfasting (*A*) and fasting (*B*) blood glucose levels, IPGTTs (*C*), AUC (*D*), total pancreatic insulin content (*E*), and plasma glucagon-to-insulin ratios (*F*) determined in the mice described in the legend for Fig. 1. All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test, $n = 6$ mice per group. □, diabetic control; ■, diabetic control with MK0431 diet. N.S., not significant.

Assessment of functional islet mass in STZ-induced diabetic mice after islet transplantation. The effect of MK0431 on islet graft survival was determined by micro-PET imaging. Mutant thymidine kinase expressed in the transplanted islets phosphorylates the substrate [^{18}F]F-HBG after systemic injection, thus trapping it in intact cells. As previously demonstrated (27,28), islet graft survival can then be quantified by PET scanning. The PET signal from transplanted islets in the normal chow diet group decreased dramatically over time after transplantation. However, the MK0431 group showed sustained PET signals for up to 4 weeks after transplantation (Fig. 6). Histological staining of kidney sections confirmed islet graft preservation in the MK0431 group at 1 month after transplantation (data not shown). PET signal intensity from the mice with differing glucose tolerance correlated well with AUC and GSIS ($R^2 = 0.86$ for AUC and $R^2 = 0.89$ for GSIS) (Fig. 7*A* and *B*) and had a slightly lower correlation with plasma glucagon

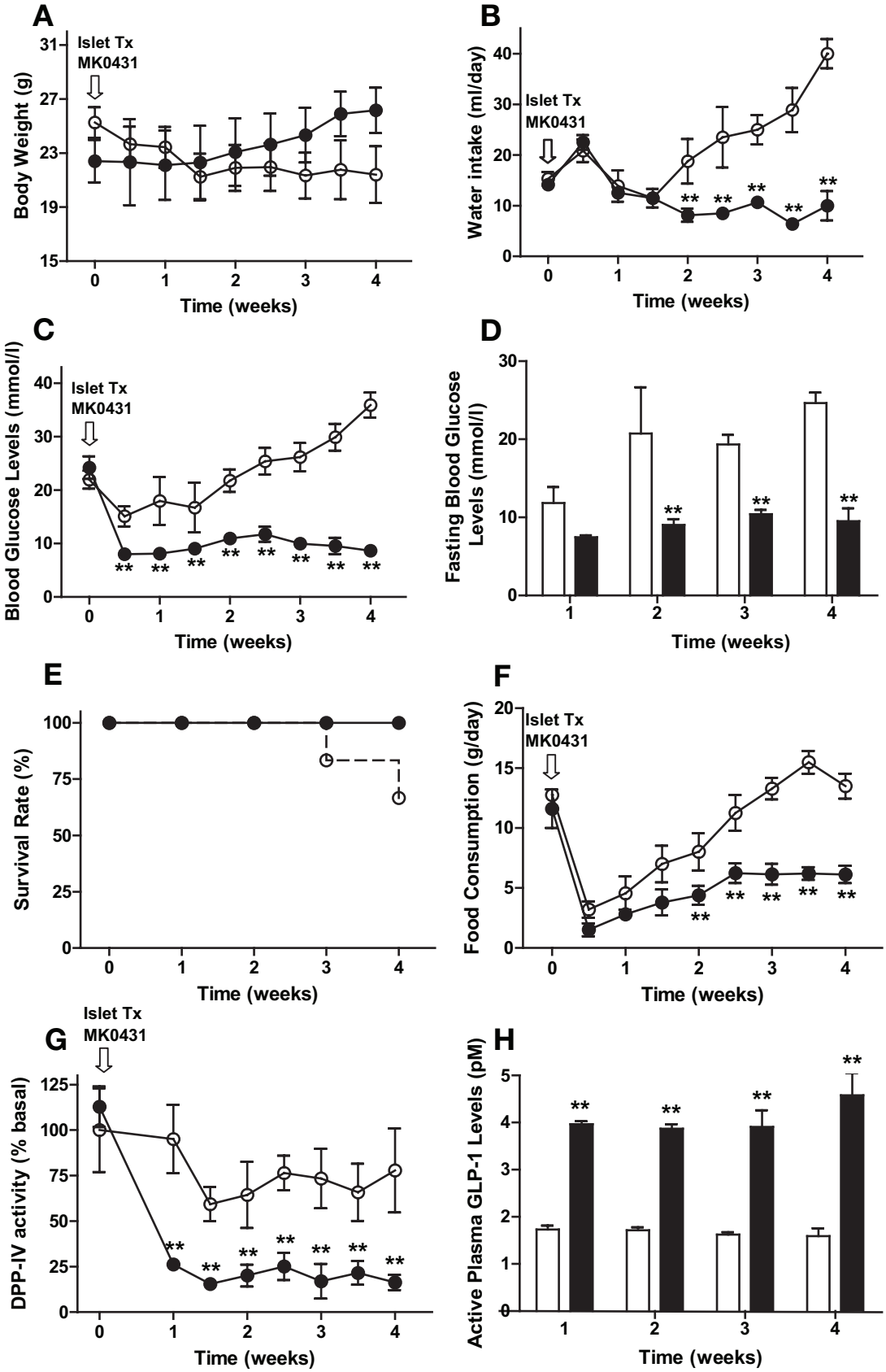

FIG. 3. The effects of MK0431 on transplanted islets. Islets isolated from nondiabetic C57BL/6 mice were infected with 250 m.o.i. of rAD-TK, 300 of which were transplanted under the right kidney capsule of STZ-induced diabetic C57BL/6 mice. After islet transplantation, mice were placed on either a normal chow diet or MK0431 diet. *A–H*: Body weight (*A*), water intake (*B*), nonfasting (*C*) and fasting (*D*) blood glucose levels, survival rate (*E*), food consumption (*F*), plasma DPP-IV activity (*G*), and plasma active GLP-1 levels (*H*) after islet transplantation. All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test, $n = 5$ mice per group. **$P < 0.05$ vs. normal chow diet mice. ○ and □, normal chow diet; ● and ■, MK0431 diet. Tx, treatment.

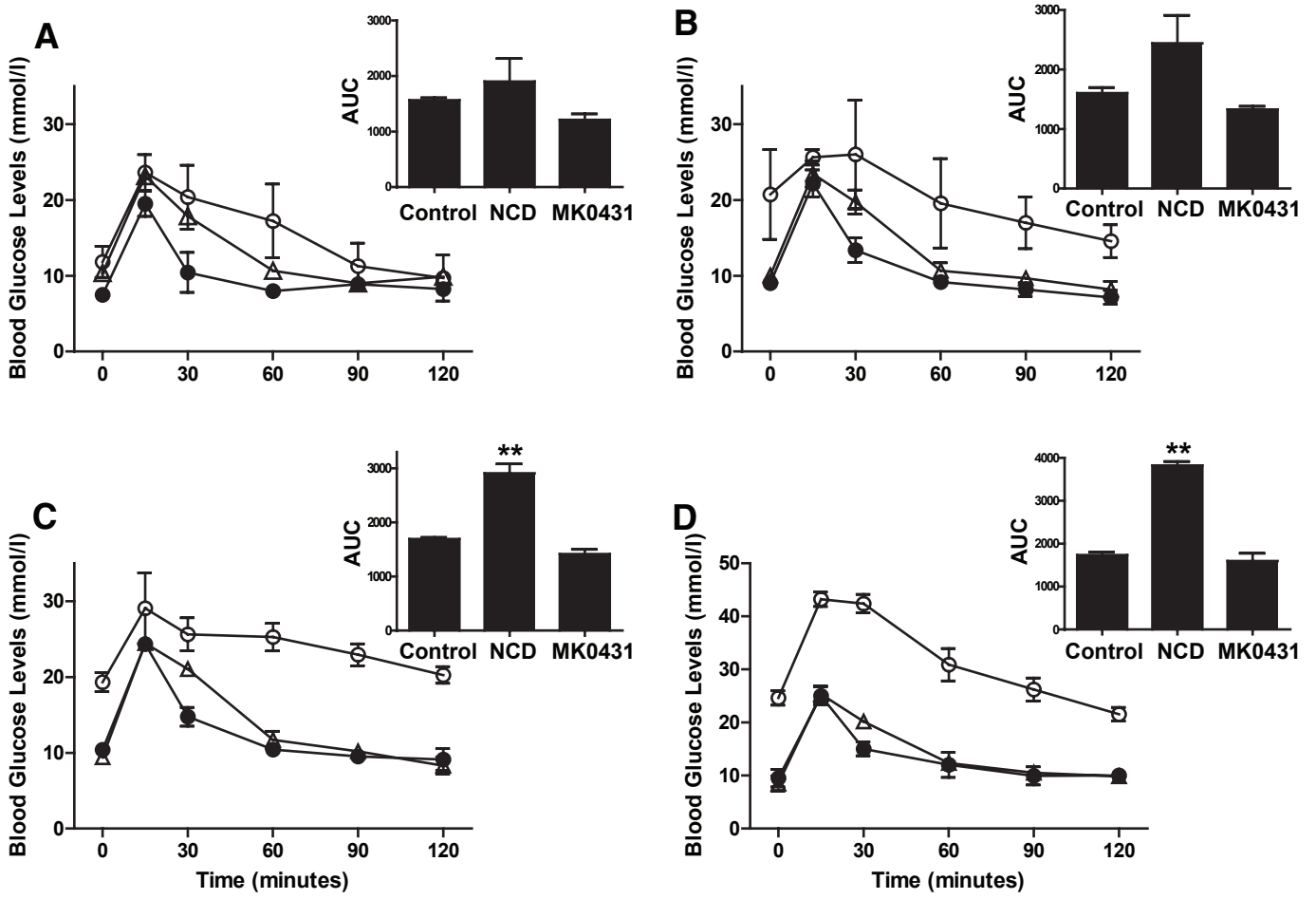

FIG. 4. Time course monitoring of glucose responses after islet transplantation. *A*: 1st week. *B*: 2nd week. *C*: 3rd week. *D*: 4th week. On the indicated days after transplantation, IPGTTs were performed on the mice described in the legend to Fig. 3, with blood glucose levels measured at 0, 15, 30, 60, 90, and 120 min after the glucose challenge (2 g/kg). All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test, n = 3–5 mice per group. **$P < 0.05$ vs. normal control mice. △, normal control mice; ○, mice placed on normal chow diet; ●, mice placed on MK0431 diet. NCD, normal control diet.

levels ($R^2 = 0.81$) and the plasma insulin-to-glucagon ratio ($R^2 = 0.74$) (Fig. 7*C* and *D*).

DISCUSSION

Multiple factors contribute to posttransplant islet loss (5–7), among which apoptosis is thought to play a major role. The incretin hormones GIP and GLP-1 both stimulate proliferation of β-cells while inhibiting apoptosis (8–12). Increasing the concentration of active incretins, by inhibiting their degradation with DPP-IV inhibitors, has been shown to be associated with β-cell preservation in animal models of type 2 diabetes (33). Additionally, in earlier studies it was shown that long-term (7-week) treatment of STZ-induced diabetic rats with the DPP-IV inhibitor isoleucine thiazolodide resulted in reduced blood glucose levels and increases in pancreatic insulin content and the number of small islets (22), suggesting that DPP-IV inhibition could preserve islets in vivo and potentially prolong islet engraftment after transplantation.

The first study was performed to establish whether similar protective effects of DPP-IV inhibition would occur in the STZ-induced diabetic mouse model used for islet transplantation. Unexpectedly, administration of STZ in mice resulted in a rapid increase in the levels of circulating DPP-IV. The reason for this is uncertain, but both the kidneys and liver express high levels of DPP-IV (34) as well as GLUT-2 (35), the transporter responsible for uptake of STZ into cells. It is therefore possible that hepatic and/or renal damage was responsible for the sustained elevation of DPP-IV activity in the control mice. Increased release of DPP-IV from the endothelium or circulating lymphocytes may also have contributed to the increase in activity. Additionally, in other animal models of type 1 diabetes, we have also observed elevated levels of circulating DPP-IV activity, suggesting that hyperglycemia and/or hypoinsulinemia may play a role. Nevertheless, MK0431 treatment resulted in a marked reduction in DPP-IV activity and enhanced active GLP-1 levels (Fig. 1*A* and *B*). However, there were no significant effects of MK0431 on body weight, water intake (Supplementary Fig. 1*B* and *C*), or fasting and nonfasting blood glucose levels (Fig. 2*A* and *B*). Additionally, blood glucose responses during the 120-min course of IPGTT in diabetic mice were unchanged by the MK0431 diet (Fig. 2*C* and *D*). The lack of a beneficial effect of the DPP-IV inhibitor is probably attributable to the low number of residual β-cells with the STZ administration protocol used. The total pancreatic insulin content in the STZ-induced diabetic mice was only 7.3 ± 0.4 ng/mg protein, <30% of that measured in the STZ rat pancreas (22), and very few β-cells were detectable by histology.

To examine the potential protective effect of DPP-IV inhibitor treatment on transplanted islets, suboptimal doses of islets were transplanted in the STZ-induced

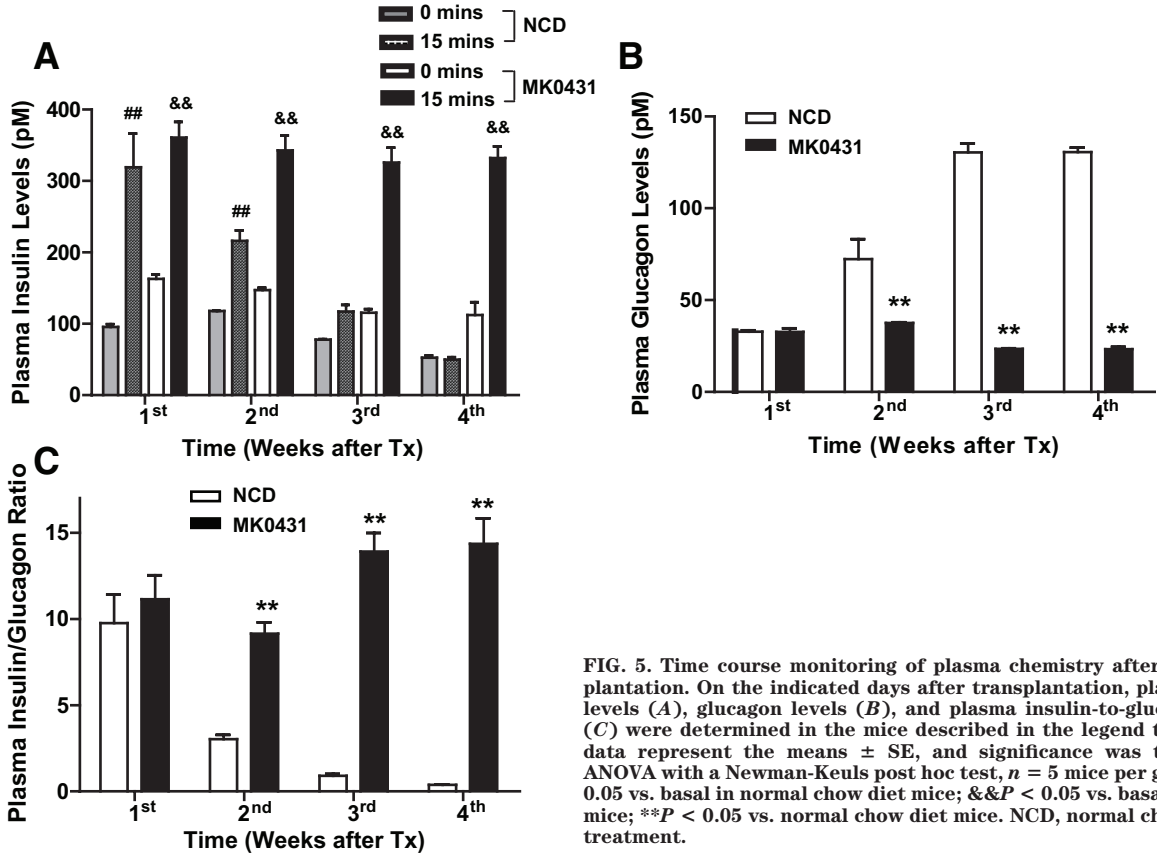

FIG. 5. Time course monitoring of plasma chemistry after islet transplantation. On the indicated days after transplantation, plasma insulin levels (*A*), glucagon levels (*B*), and plasma insulin-to-glucagon ratios (*C*) were determined in the mice described in the legend to Fig. 4. All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test, $n = 5$ mice per group. ##$P <$ 0.05 vs. basal in normal chow diet mice; &&$P < 0.05$ vs. basal in MK0431 mice; **$P < 0.05$ vs. normal chow diet mice. NCD, normal chow diet; Tx, treatment.

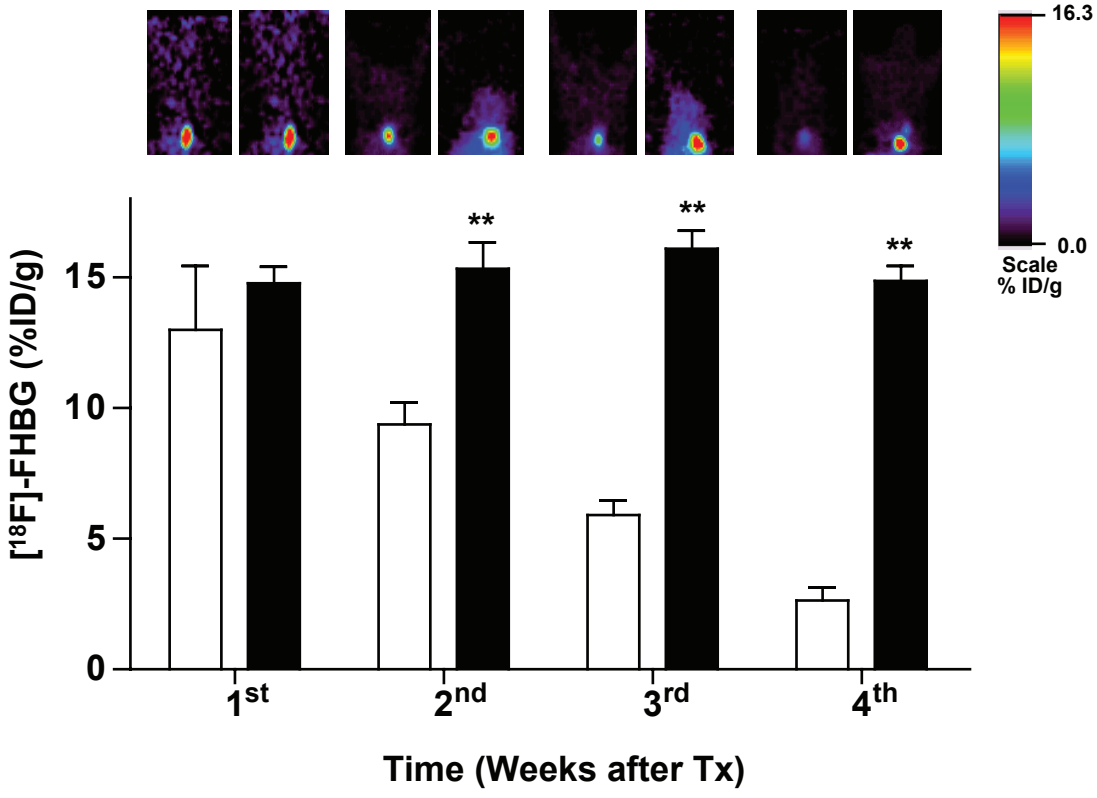

FIG. 6. Time course monitoring of MicroPET signals detected from recipient diabetic mice after islet transplantation. Mice described in the legend to Fig. 4 were injected with [^{18}F]FHBG on the indicated days after transplantation and scanned. ROIs were placed on the kidney area of the microPET image, and two peak values of % ID/g from the two time activity curves for each region were used for determination of the signals. All data represent the means ± SE, and significance was tested using ANOVA with a Newman-Keuls post hoc test, $n = 5$ mice per group. **$P < 0.05$ vs. normal chow diet mice. □, normal chow diet; ■, MK0431 diet. Tx, treatment.

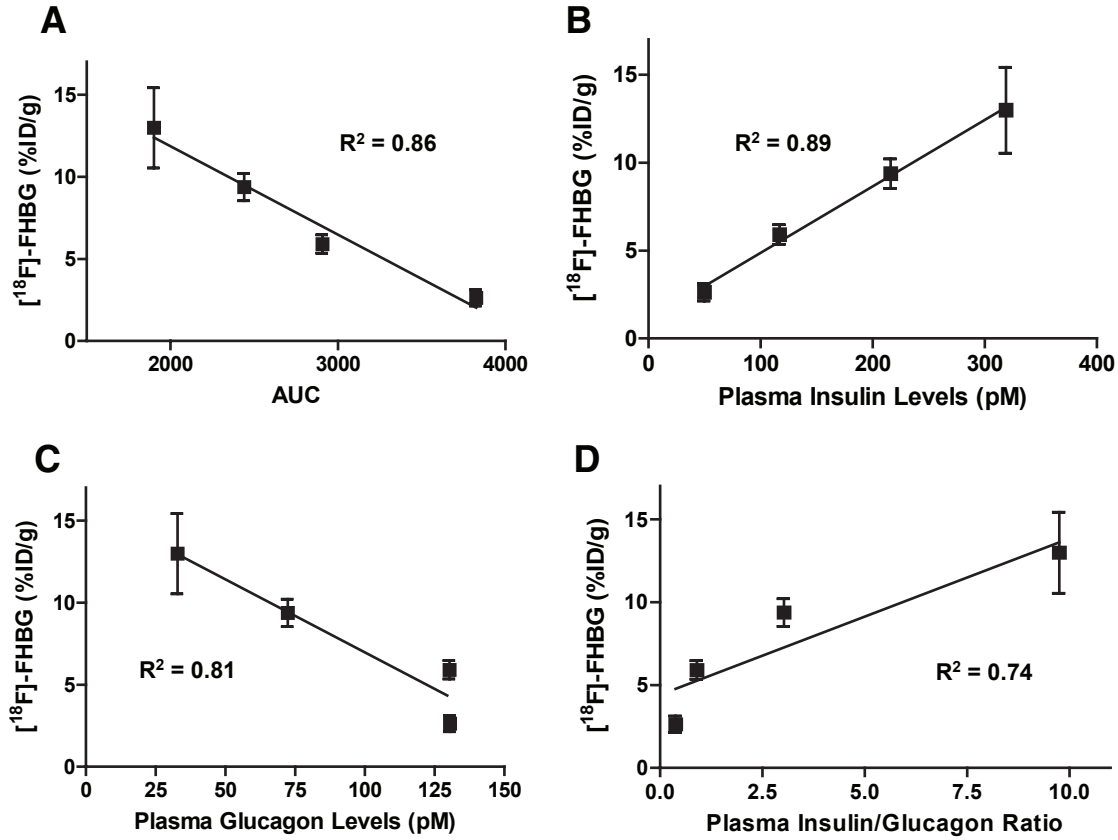

FIG. 7. Correlation between PET signals and metabolic tests. Correlation between PET signals and AUC (*A*), GSIS (*B*), plasma glucagon levels (*C*), and plasma insulin-to-glucagon ratios (*D*). Data were analyzed using the linear regression analysis program PRISM.

diabetic mice. Although there is considerable variation in the number of islets reported to achieve euglycemia among different studies, we and others (32) have found that 300 islets is generally suboptimal for C57BL/6 mice, resulting in transplanted mice that are very sensitive to the effects of treatments that either prolong or decrease islet graft survival. In pilot studies, mice that received transplants of lower numbers of islets (100) remained hyperglycemic and did not benefit from treatment with MK0431 (data not shown).

The body weight in MK0431-fed mice increased by 4 g over the study period, whereas the normal chow diet –fed mice lost weight. Nonfasted blood glucose levels in normal chow diet–fed mice were decreased by ~15 mmol/l at 3 days after islet transplantation, but levels increased to >30 mmol/l by 4 weeks (Fig. 3*C*). Fasting blood glucose followed a similar trend (Fig. 3*D*), reflecting the suboptimal nature of the islet transplantation. By contrast, fasting and nonfasting blood glucose in islet-transplanted diabetic mice on the MK0431 diet decreased to <10 mmol/l and remained so throughout the study period (Fig. 3*C* and *D*). IPGTTs confirmed that the transplanted islets in the normal chow diet–fed group lost their capacity to regulate blood glucose levels over time (Fig. 4), whereas the MK0431-fed group showed preserved ability to regulate blood glucose levels until the end of the study with sustained GSIS and plasma insulin-to-glucagon ratios (Figs. 4 and 5), suggesting prolongation of islet graft survival. The overall improvement in metabolic status of the MK0431-treated mice was also reflected in the stable water intake (Fig. 3*B*) and much lower food consumption than the normal chow diet–fed mice (Fig. 3*F*), accompanied by an increase in body weight of the former group.

Although the metabolic studies indicated that MK0431 promoted islet survival, it was not possible to unambiguously discriminate between maintenance of islet mass and improved islet function. Although high correlations between metabolic determinations and independent measures of β-cell function have been obtained (36), assessment of functional islet mass through imaging can directly provide quantitative information on islet graft status. Additionally, such techniques are directly applicable for preclinical studies on drugs with the potential for improving graft survival. Magnetic resonance imaging, optical imaging, and PET all offer their own advantages. PET is a noninvasive metabolic imaging modality that has been extensively used to study biochemical and biological process in vivo. With the PRG (PET reporter gene)/PRP (PET reporter probe) system, based on a mutant form of herpes simplex virus 1 thymidine kinase (*HSV1-sr39tk*), the PET signal is directly proportional to the enzymatic activity of sr39TK (37–38). Recently, we (27–28) and others (39–40) showed that microPET imaging is directly applicable to the quantification of islet transplants. In the current study, the effect of MK0431 on functional islet mass was quantified by PET imaging throughout the 4 weeks, and there was a profound prolongation of the PET signal in the inhibitor-treated mice (Fig. 6). PET signal intensity also showed a strong correlation with AUC or GSIS in the IPGTTs (Fig. 7), indicating that oral administration of MK0431 exerted cytoprotective effects on the transplanted islets.

In conclusion, the DPP-IV inhibitor MK0431, acting at least partially through increasing the circulating concentrations of active incretin hormones, exerted protective effects resulting in the prolongation of islet graft survival.

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