Caspase Inhibitor IDN6556 Facilitates Marginal Mass Islet Engraftment in a Porcine Islet Autotransplant Model

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Background. Large numbers of islets are lost in the early phase after clinical islet transplantation, through apoptosis, necrosis, or innate inflammatory injury. We previously demonstrated the efficacy of a series of caspase inhibitors in mouse models on islet engraftment through reduction in early posttransplant apoptosis. We studied IDN6556, a caspase inhibitor with a first-pass effect, in a large animal (pig) intraportal marginal mass islet autotransplant model.
Methods. Total pancreatectomy and marginal mass islet autotransplantation were carried out in Yucatan miniature swine to explore the effects of IDN6556 on islet engraftment. Pigs were treated with IDN6556 at a dose of 20 mg/kg orally twice daily (n=7) or phosphate-buffered saline control (n=6) orally for 7 days, and blood glucose was monitored for 1 month. Glucose tolerance and acute insulin release were determined at 1 month.
Results. There were no differences in islet procurement, isolation, or islet functional parameters between the two groups. Pigs receiving IDN6556 had lower fasting blood glucose level after transplantation and a higher percentage (100% vs. 33.3%) showed fasting blood glucose levels less than 11 mM. This translated into an enhanced metabolic reserve and acute insulin release for pigs in the treatment group.
Conclusions. IDN6556 led to enhanced islet engraftment in this large animal islet transplant model. Although this study has limitations including a short interval of study (1 month) and the use of unpurified islets, the results justify early clinical trials of IDN6556 in islet transplantation.

Keywords: Islets, IDN6556, Engraftment, Caspase inhibitor.

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Islet transplantation has become a promising treatment option for select patients with type 1 diabetes. Since 2000, there have been an estimated 700 islet allotransplantations performed worldwide, with combined results of three leading centers reporting insulin independence rates of 82% at 1 year (*1–3*). Most centers, but not all, report the need for more than one donor to achieve sufficient islet engraftment mass, and rates of insulin independence have waned over time, with 55% remaining insulin-free at 2 years and as low as 15% by 5 years (*4*). The current paradigm of success has been to achieve sufficient human C-peptide secretion to protect from hypoglycemia and correct hemoglobin A_{1c}, even if this falls short of complete insulin independence. A major goal, therefore, is to augment the initial islet engraftment mass and provide sustained immunologic protection from autorejection and allorejection, and several promising strategies have emerged to promote this. It is known that up to 70% of the transplanted islet mass is lost within the initial hours to days after transplantation (*5–9*). Blood-mediated inflammatory reaction, hypoxia, delayed revascularization, reperfusion, and inflammatory cytokines all contribute to early islet loss, culminating in islet apoptosis or necrosis. This process is initiated in the donor, exacerbated during cold ischemic storage and during islet isolation and subsequent culture, and persists during the early engraftment phase in the recipient portal venous system (*5*, *10–13*).

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IDN6556 was provided free of charge through a material transfer agreement from Pfizer, Inc, USA, and, more recently, from Conatus Pharmaceuticals, Inc, San Diego, CA.
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In the present study, we investigate the potential protective effect of a potent pan-caspase inhibitor, IDN6556 (formerly Pfizer, Inc, and recently Conatus Pharmaceuticals, Inc, San Diego, CA), as a means to prevent apoptosis of intraportal islet transplants in a porcine autograft large animal model. We previously reported the potency of this compound in protecting syngeneic marginal mass in intraportal islet transplants in mice and similarly with human islets transplanted in immunodeficient mice (*14*).

RESULTS

IDN6556 Substantially Increases Rates of Euglycemia With Marginal Mass Islet Autotransplantation

Complete surgical pancreatectomy led to absence of stimulated insulin response in all treated animals (treatment and controls), confirming effective surgical resection of the beta-cell mass (Table 1). There was no difference in the mean acute insulin release (AIR_{ARG}) immediately after pancreatectomy between the two groups. The cold ischemic time was short (approximately 30 min) and not different between groups (Table 1). Islet isolation was successful in all cases, with only a marginal mass returned to each pig (1000–1500 islet equivalents [IE]/kg). This equated to at least 49% of the islet mass (range, 48%–83%) being discarded, and this did not differ between groups. There was no difference in islet viability or islet score between groups (Table 1).

Figure 1(A) shows the mean daily fasting blood glucose excursions for the IDN6556 intervention and control groups; the marginal mass led to early hyperglycemia in both groups, but in the IDN6556 group, glycemic control improved markedly after day 12 (day 16: mean blood glucose, 11.02 vs. 18.8 mM, $P<0.05$; day 23: mean blood glucose, 10.1 vs. 20.2 mM, $P<0.05$). Figure 1(B) displays the dosage of insulin used each day, confirming that differences in fasting blood glucose were not due to the disproportionate insulin usage. The proportion of pigs maintaining blood glucose levels less than 11 mM after marginal mass islet transplantation was significantly higher in the IDN6556 treatment group compared with that in the vehicle-treated control group (100% IDN6556 vs. 33.3% control, $P<0.05$ by log-rank analysis; Fig. 1C).

TABLE 1. Comparison of pancreatectomy and islet isolations

	Control (n=6)	IDN6556 (n=7)	*P*
Pig weight,[a] kg	26.43	23.71	0.35
Pancreas cold ischemic time, min	32.86	29.57	0.67
Islet mass isolated per kilogram body weight, IE/kg	4112	3749	0.58
Islet mass isolated per gram pancreas weight, IE/g	2833	2741	0.87
Islet viability, %	91.2	92.3	0.71
Islet score	5.9	5.9	0.90
AIR_{ARG}[c] after pancreatectomy, ng/mL	0.036	0.022	0.51
Mean islet mass infused, IE/kg	1167	1214	0.75

[a] At the time of pancreatectomy.
[b] A measure of islet quality.
[c] Acute insulin release in response to arginine.

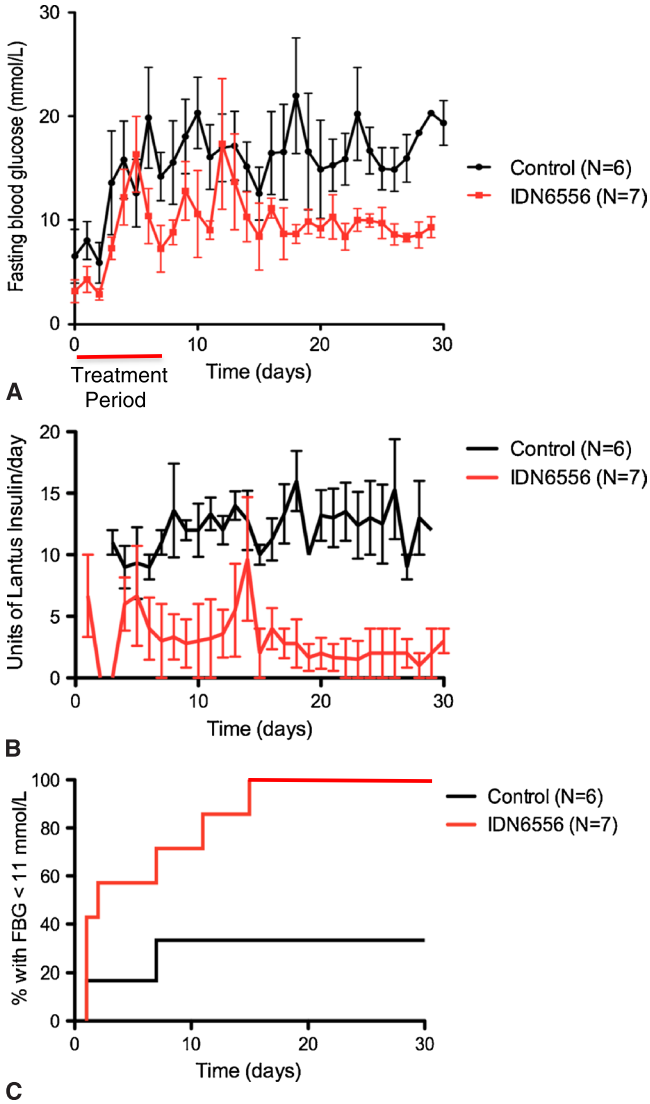

FIGURE 1. Fasting blood glucose and insulin administration. Female Yucatan swine underwent total pancreatectomy and marginal mass islet autotransplantation. For 1 week after transplantation, pigs were treated with either the caspase inhibitor IDN6556 (20 mg/kg orally twice daily, n=7) or vehicle (PBS, n=6). (A) Fasting blood glucose values were collected daily. (B) Units of Lantus insulin administered to pigs in each of the groups. (C) Percentage of pigs with persistent blood glucose levels less than 11 mM as a function of time after transplantation ($P<0.05$ by log-rank analysis).

Islet Potency Was Not Different Between Groups

Islet aliquots from the discarded portion of each isolation were assessed in triplicate for insulin release in response to glucose. Figure 2(A) demonstrates that there was no significant difference in the in vitro potency of islets

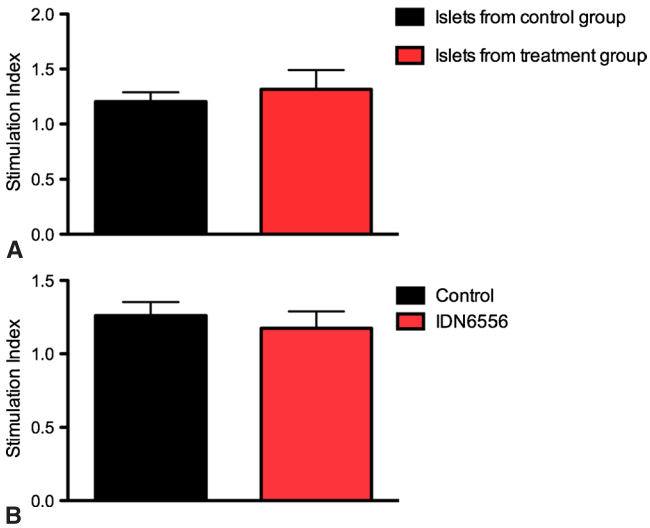

FIGURE 2. In vitro assessment of insulin secretory function of isolated islets. Triplicate islet aliquots from each islet isolation were set aside for static assay analysis. Islets were washed in glucose-free medium (CMRL) followed by incubating in either low- (2.8 mM) or high- (20 mM) glucose medium for 1 hr at 37°C. Supernatants were analyzed for insulin content. The stimulation index was calculated as the ratio of insulin release in high- to low-glucose medium. There was no significant difference in stimulation index between islets isolated from IDN6556-treated pigs and those isolated from vehicle-treated pigs (A; *t* test, $P>0.05$). For each islet isolation, separate triplicate aliquots were set aside and treated as above except for the addition of IDN6556 or PBS (control) to all media (B). There was no difference in the stimulation index between islets incubated in the caspase inhibitor and those with PBS (*t* test, $P>0.05$).

isolated from pigs in the treatment group versus those in the control group based on the insulin stimulation index (IDN6556 vs. control, 1.31 vs. 1.21; unpaired *t* test, $P>0.05$). The stimulation index reflects the ratio of insulin release in islets exposed in vitro to low and high glucose.

IDN6556 Is Not Toxic to Islets In Vitro and Does Not Alter Insulin Release

To determine any detrimental impact of the drug IDN6556 on islets in vitro, the stimulation index was further assessed in triplicate in control islets maintained in culture for 2 hr versus in the presence of 100 μM IDN6556 (Fig. 2B). There was no significant difference in the stimulation index between groups (IDN6556 vs. control, 1.18 vs. 1.26; paired *t* test, $P>0.05$).

IDN6556 Treatment Improves Metabolic Reserve in Marginal Mass Islet Transplants

One month after islet autotransplantation, pigs were fasted overnight and subjected to arginine-potentiated glucose tolerance testing. In the IDN6556 treatment group, glucose tolerance did not differ significantly from the normal naive pigs (preoperative controls, n=13; Fig. 3A). This improved glucose tolerance translated into an improved area under the curve when comparing IDN6556-treated and control (vehicle) pigs (unpaired *t* test, $P<0.05$; Fig. 3B). Similarly, the area under the curve did not differ between IDN6556 treatment and baseline control normal pigs, demonstrating that the marginal engrafted islet mass, protected by IDN6556, was sufficient to provide normal metabolic reserve (unpaired *t* test, $P>0.05$).

In response to arginine potentiation (Fig. 4), AIR_{ARG} in the IDN6556 group was significantly higher than that in the control (vehicle) group (IDN6556 vs. control, 0.30 vs. 0.16 ng/mL; unpaired *t* test, $P<0.05$).

IDN6556 Did Not Lead to Systemic Toxicity in Pigs

We compared body weight, activity, and signs of toxicity between groups and identified no evidence for systemic toxicity in the IDN6556-treated group when given at a dose of 20 mg/kg twice daily for 7 days. Formal toxicology studies were not conducted because there are clinical data demonstrating safety and lack of toxicity. There was no mortality

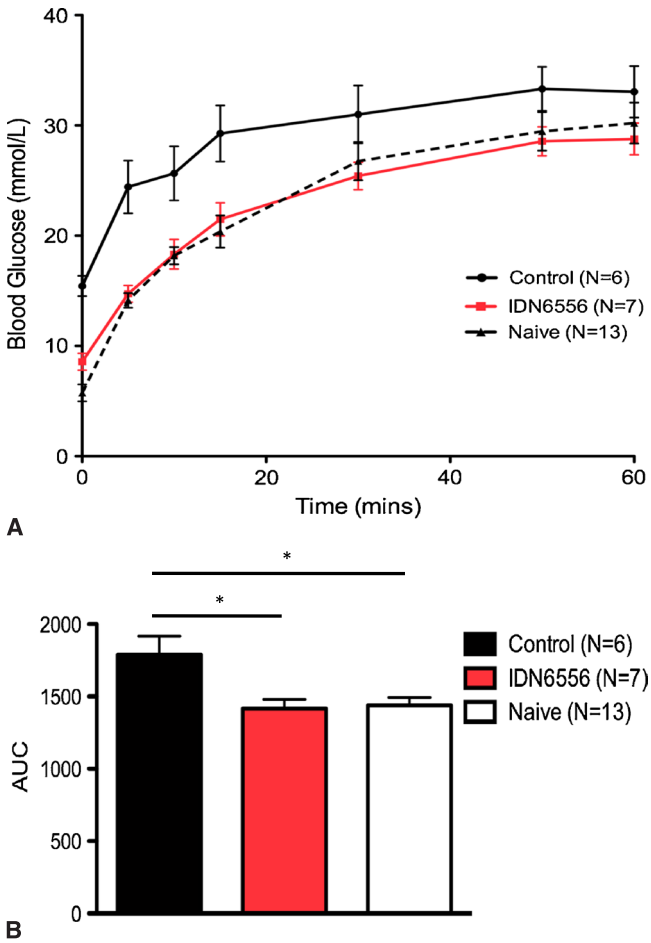

FIGURE 3. Glucose tolerance 1 month after islet transplantation. Pigs were fasted overnight and given a 500-mL intravenous infusion of 20% dextrose for 1 hr. (A) Blood glucose was sampled throughout the hour. All pigs underwent a similar procedure 1 week before pancreatectomy (naive). (B) Area under the curve (AUC) values. Pigs treated with IDN6556 for 1 week after transplantation displayed improved glucose tolerance compared with vehicle-treated pigs (*$P<0.05$ for AUC values). There was no difference between the glucose tolerance of naive and IDN6556-treated pigs 1 month after marginal mass islet transplantation ($P>0.05$).

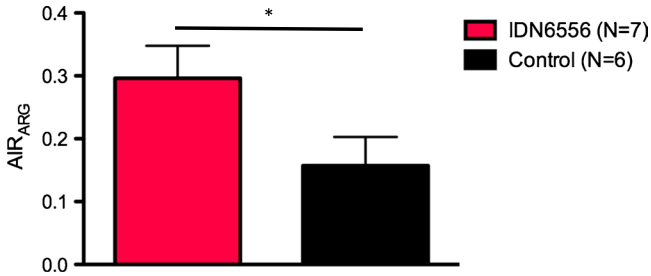

FIGURE 4. Acute insulin release in response to arginine. At 1 month, immediately after the dextrose infusion/glucose tolerance test, a 5-g bolus of arginine was administered intravenously. Serum was sampled during the following 10 min to determine porcine insulin release. Pigs treated with the caspase inhibitor IDN6556 displayed a significantly higher AIR_{ARG} compared with vehicle-treated pigs (*t* test, $*P<0.05$).

related to IDN6556 treatment. Terminal laparotomies at 1 month revealed no evidence of infection or malignancy.

DISCUSSION

This study of unpurified, intraportal islet transplantation in a large animal (pig) autograft model confirms our previous findings and hypothesis that apoptosis remains a dominant target for islet loss in the early posttransplant period and that prevention of early apoptosis can substantially augment islet engraftment mass. We have previously investigated caspase inhibitors as a means of preventing islet apoptosis; zVAD-FMK and EP1013 led to enhancement of islet engraftment in mouse models of diabetes both using marginal mass syngeneic grafts and using human islets (*15*, *16*). In marginal mass transplants, we were consistently able to reduce the minimum islet implant mass to less than 30% compared with control transplants when different caspase inhibitors were given for up to 10 days after transplant in mice, when transplanted with syngeneic islets or human islets transplanted into immunodeficient mice (*15*, *16*). The mechanism underlying this was predominantly reduced apoptosis as measured by terminal deoxynucleotide transferase-mediated dUTP nick-end labeling observed at 24 hr after transplant.

The fluoromethylketone group in zVAD-FMK has potential toxicities and is unsuitable for clinical application; EP1013 is no longer accessible as the company Epicept folded. In seeking a potent pan-caspase inhibitor for potential clinical translation, we identified IDN6556 (3-[2-(2-*tert*-butylphenylaminooxalyl)-amino]-propionylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid) as a more suitable compound because it has already been tested as a liver-targeted apoptosis inhibitor and used previously together with clinical immunosuppression (*17*). When tested in mice, the drug was well tolerated and was not associated with either detrimental islet toxicity or systemic toxicity, and the groups treated with IDN6556 demonstrated marked improvement in islet engraftment, with sustained function over time for prolonged periods after discontinuation of the agent (*14*). The aim of the current study was to further explore IDN6556 in a large animal (pig) marginal mass islet transplantation model. IDN6556 was administered orally in the present study to take potential advantage of first-pass portal adsorption and direct protection of the intraportal islet mass (*18*).

We confirmed that complete surgical pancreatectomy led to a diabetic state, with absent acute insulin release. We observed substantial improvement in the engraftment of marginal mass, unpurified pig islets with 7-day treatment with IDN6556, with improved glycemic control, and metabolic control that resembled the normal, naive pig profile. There was no intention to treat the control group different from the treatment group with respect to posttransplant glycemic control. Although we acknowledge that there were minor differences in mean glycemic control between the control and treatment groups in the first 2 days after transplant, this did not reach statistical significance. A marginal mass of 1000 to 1500 IE/kg unpurified islets was sufficient to reverse diabetes in all cases (7/7) in the IDN6556 treatment group but was sufficient in only two of six cases in the control group. Approximately half of the isolated islet mass was discarded in both groups—therefore, one pancreas may potentially have been sufficient to treat two recipients in the IDN6556 group (not tested in the autograft state).

Oral administration of IDN6556 therefore seems to be effective in first-pass intraportal adsorption and direct islet protection in this large animal model. In our previous reports, we used intraperitoneal dosing in mice (*14–16*). It is possible that more prolonged or increased oral dosing may provide even further protection, but this was not tested in the present study.

The current practice in clinical islet transplantation is to use purified islet products in the setting of allotransplantation. In autotransplantation, the practice is different; unpurified or partially purified tissue is used to maximize the engraftment of a limited tissue mass. We did not purify the islet preparations in this particular experiment because we wished to explore the potency of unpurified islet intraportal preparations of marginal mass islets in this pig model. We recognize this as a potential confounding variable. We would consider the use of marginal mass, marginal volume of unpurified islet tissue for future clinical intraportal transplantation, especially if the islet volume was limited to less than 10 mL, under conditions of therapeutic heparinization and with careful intermittent monitoring of portal pressure. This would likely be relatively safe but could lead to an increased risk of peripheral segmental portal branch occlusion.

In terms of mechanism of protection, we have consistently found previously that apoptosis was reduced with pan-caspase inhibitors, including IDN6556 in mouse and human islets transplanted into mice, based on terminal deoxynucleotide transferase-mediated dUTP nick-end labeling assays (*14*). However, we did not repeat liver biopsies at 24-hr time points in the present study because this would have required more large animals, and repeated surgical interventions were not believed to be appropriate. We also did not specifically measure cytokine or chemokine release in the early posttransplant period. It is possible that IDN6556 may lessen cytokine release through the inhibition of exocrine cell apoptosis in these unpurified islet preparations.

We identified no evidence of islet toxicity from systemic IDN6556 treatment and similarly found no signs of islet toxicity in vitro. In terms of systemic toxicity, the current

study was not designed to provide formal toxicology data, but specifically we found no associated mortality, morbidity, or specific toxicity from IDN6556 at 20 mg/kg twice daily in pigs. Terminal laparotomies revealed no signs of malignancy or infection, but clearly the period for observation is limited. Clinical phase 1/2 dose escalation and dosing of IDN6556 for up to 12 weeks in subjects with underlying hepatitis C infection failed to demonstrate increased rates of malignancy or toxicity and led to improved rates of hepatocellular protection in the presence of hepatitis C (*19*, *20*). Furthermore, there seemed to be no synergistic toxicity when IDN6556 was combined with standard immunosuppression in patients undergoing liver transplantation, when IDN6556 was added both to the preservation flush solution and to subjects at a dose of 0.5 mg/kg intravenously for 24 hr after transplant, and the compound reduced apoptotic hepatocellular injury (*17*). Previous studies showed clear evidence of hepatoprotection in rodents (*17*, *21–23*).

Limitations of the present study include (*a*) small numbers of large animals (limited based on cost, capacity for large animal housing, and ethical considerations); (*b*) use of unpurified islets, making the accuracy of islet mass quantification less precise, owing to the transplantation of islet fragments or islets ''cuffed'' in exocrine tissue; and (*c*) the observation period being limited to 1 month after transplant. Therefore, although the present findings are encouraging, it remains to be seen whether similar positive findings will be replicated in clinical patients treated with IDN6556. Currently, clinical studies are in development to test the effect of this compound in patients with type 1 diabetes receiving purified intraportal islet transplants.

In summary, the pan-caspase inhibitor, IDN6556, improved marginal mass intraportal islet engraftment in a pig islet autotransplant model and, combined with the previous similar findings in mouse and human islets transplanted in mice, now provides compelling support for further investigation in the clinic.

MATERIALS AND METHODS

Animals

Adult female Yucatan miniature swine (22–32 kg) were obtained from the colony at Memorial University, St John, Newfoundland. Ethical approval was obtained from the animal welfare committee at the University of Alberta, and all animals were maintained and cared for according to the Canadian Council on Animal Care guidelines.

Caspase Inhibitor

IDN6556 was a generous gift from Pfizer, Inc (New York, NY) in powder form and was stored at room temperature. Animals received either IDN6556 20 mg/kg orally in phosphate-buffered saline (PBS; n=7, treatment group) or PBS control (n=6, control group) twice daily for 7 days after transplantation, beginning on the first postoperative day. There was random assignment to groups. For the in vivo portion of the studies, IDN6556 was only given orally, initially by gavage, and then mixed with the solid diet. IDN6556 was not administered during islet culture in the in vivo studies because islets were transplanted in an unpurified state shortly after completion of the isolation process.

Pancreatectomy and Islet Transplantation

Animals were fasted more than 12 hr before surgery. After induction of anesthesia, endotracheal intubation, antibiotic prophylaxis (cefazolin; Novopharm, Toronto, Ontario, Canada; 1 g intramuscularly) and central venous line access, physiological monitoring and intravenous fluid support, the abdomen was entered through a midline laparotomy, and a harmonic scalpel (Ethicon, Markham, Ontario, Canada) used to provide meticulous dissection for a total pancreatectomy. The major pancreatic duct was cannulated using a 24-gauge catheter with the pancreas in situ to facilitate pancreas distension during the digestion phase. Shortly after pancreatectomy, a 5-g intravenous bolus of arginine-HCl was administered, and serum samples were collected at 0, 2, 3, 4, 5, 7, and 10 min to confirm absence of insulin secretion and complete removal of the entire β-cell mass. After islet isolation, an unpurified marginal mass of islets was gravity infused into a tributary of the portal vein through a 7F catheter. We did not directly measure the packed cell volume infused, although this was less than 10 mL in all cases. After appropriate titration, and from previous studies in our laboratory, we found a marginal islet mass of 1000 to 1500 IE/kg to be the ideal mass to delineate differences between the control and treatment groups (*24*). A 70-U/kg bolus of heparin was administered with the islets. Before closure, the duodenum was checked for viability in all cases.

Posttransplant Care

Central access and intravenous fluids were maintained for 48 hr after surgery, and then pigs were fed a clear fluid diet for 2 days returned to standard pig chow supplemented with pancreatic enzyme replacement (Pancrease-V; Bioniche Animal Health Canada, Inc, Belleville, Ontario, Canada). Blood glucose was monitored each morning using a glucometer (One Touch Ultra; Johnson & Johnson, Skillman, NJ), and Lantus insulin was titrated as needed to maintain blood glucose levels of 11 mM or less. Low-molecular-weight heparin was administered for 1 week after transplant (Lovenox 1.5 mg/kg subcutaneously daily; Sanofi Aventis, Laval, Quebec, Canada). Pigs were weighed twice weekly. Opioid and nonsteroidal anti-inflammatory analgesia was provided with buprenorphine (Schering-Plough, Hertfordshire, United Kingdom) twice daily and meloxicam daily (Boehringer Ingelheim, Burlington, Ontario, Canada) for 5 days after surgery.

Islet Isolation

The pancreas was syringe distended intraductally with cold enzyme solution (Liberase DL; Roche Applied Science, Mannheim, Germany) through the previously placed catheter. Digestion of the pancreas was conducted at 37°C in a Ricordi chamber; (Biorep Technologies, Miami, FL); time was dependent on free islets appearing in a 1-mL withdrawn sample. The mean dilution time was 19.27 ± 3.9 min. Samples were stained with dithizone (Sigma-Aldrich, Oakville, Ontario) (1:10 in PBS) before viewing and were counted by normalizing to a standard islet size using islet equivalents (IE) of 150 μm. Viability was assessed with SYTO green (SYTO-13; Molecular Probes, Eugene, OR) and ethidium bromide (Sigma-Aldrich). Cells that stained green were considered viable, whereas ethidium bromide stained dead or damaged tissue (*25*, *26*).

In Vitro Testing of Islet Toxicity With IDN6556 in Glucose-Stimulated Insulin Release Assays

Triplicate aliquots containing 1000 IE were washed in glucose-free medium (CMRL supplemented with 10% fetal calf serum [FCS]). Medium was then replaced with either low-glucose medium (CMRL containing 2.8 mM D-glucose and supplemented with 10% FCS) or high-glucose medium (CMRL containing 20 mM D-glucose supplemented with 10% FCS) and incubated for 1 hr at 37°C under 5% CO_2. A second triplicate was washed and incubated in similar medium with the addition of the caspase inhibitor IDN6556 (100 μM). Aliquots from the supernatants were analyzed for porcine insulin content using a radioimmunoassay (Linco Research, St Charles, MO). In each instance, the fold stimulation was calculated by dividing the insulin values released from islets in high-glucose medium by that of islets in low-glucose medium run in parallel.

In Vivo Glucose Potentiation of Arginine-Induced Secretion

Glucose potentiation of arginine-induced secretion was performed 1 week before transplantation (n=13, naive group) and at 1 month after transplant. Animals were fasted overnight and placed under general anesthesia, and central venous access was secured. Protocols for glucose potentiation of

arginine-induced secretion are as described previously (*24*). Briefly, blood samples were drawn at 5, 10, 15, 30, 50 and 60 min and then a 5-g bolus of arginine-HCl was given, with further sampling at 62, 63, 64, 65, 67, and 70 min. Blood glucose was measured glucose oxidase in standard assay, and porcine insulin was analyzed simultaneously with a sensitive radioimmunoassay kit (limits of sensitivity, 0.02–1.0 ng/mL insulin; Linco Research). The AIR_{ARG} was calculated from the three peak serum insulin levels at 2, 3, 4, and 5 min after arginine and subtracting the basal serum insulin level.

Statistical Analysis

Data were analyzed using GraphPad Prism (version 5.0c; GraphPad Software, Inc, San Diego, CA). $P<0.05$ was considered statistically significant. Results are expressed as mean ± SEM.

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REFERENCES

1. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343: 230.
2. The Collaborative Islet Transplant Registry 2009 Annual Report. Available at: *http://www.citregistry.org/reports/reports.htm*.
3. Shapiro AM, Ricordi C, Hering B. Edmonton's islet success has indeed been replicated elsewhere. *Lancet* 2003; 362: 1242.
4. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; 54: 2060.
5. Biarnes M, Montolio M, Nacher V, et al. beta-Cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 2002; 51: 66.
6. Berney T, Mamin A, James Shapiro AM, et al. Detection of insulin mRNA in the peripheral blood after human islet transplantation predicts deterioration of metabolic control. *Am J Transplant* 2006; 6: 1704.
7. Davalli AM, Ogawa Y, Ricordi C, et al. A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation* 1995; 59: 817.
8. Eich T, Eriksson O, Lundgren T. Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. *N Engl J Med* 2007; 356: 2754.
9. Eriksson O, Eich T, Sundin A, et al. Positron emission tomography in clinical islet transplantation. *Am J Transplant* 2009; 9: 2816.
10. Paraskevas S, Maysinger D, Wang R, et al. Cell loss in isolated human islets occurs by apoptosis. *Pancreas* 2000; 20: 270.
11. Davalli AM, Scaglia L, Zangen DH, et al. Vulnerability of islets in the immediate posttransplantation period. Dynamic changes in structure and function. *Diabetes* 1996; 45: 1161.
12. Moritz W, Meier F, Stroka DM, et al. Apoptosis in hypoxic human pancreatic islets correlates with HIF-1alpha expression. *FASEB J* 2002; 16: 745.
13. Contreras JL, Eckstein C, Smyth CA, et al. Brain death significantly reduces isolated pancreatic islet yields and functionality in vitro and in vivo after transplantation in rats. *Diabetes* 2003; 52: 2935.
14. McCall M, Toso C, Emamaullee J, et al. The caspase inhibitor IDN-6556 (PF3491390) improves marginal mass engraftment after islet transplantation in mice. *Surgery* 2011; 150: 48.
15. Emamaullee JA, Stanton L, Schur C, et al. Caspase inhibitor therapy enhances marginal mass islet graft survival and preserves long-term function in islet transplantation. *Diabetes* 2007; 56: 1289.
16. Emamaullee JA, Davis J, Pawlick R, et al. The caspase selective inhibitor EP1013 augments human islet graft function and longevity in marginal mass islet transplantation in mice. *Diabetes* 2008; 57: 1556.
17. Baskin-Bey ES, Washburn K, Feng S, et al. Clinical trial of the pan-caspase inhibitor, IDN-6556, in human liver preservation injury. *Am J Transplant* 2007; 7: 218.
18. Shapiro AM, Gallant HL, Hao EG, et al. The portal immunosuppressive storm: relevance to islet transplantation? *Ther Drug Monit* 2005; 27: 35.
19. Pockros PJ, Schiff ER, Shiffman ML, et al. Oral IDN-6556, an anti-apoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. *Hepatology* 2007; 46: 324.
20. Shiffman ML, Pockros P, McHutchison JG, et al. Clinical trial: the efficacy and safety of oral PF-03491390, a pancaspase inhibitor—a randomized placebo-controlled study in patients with chronic hepatitis C. *Aliment Pharmacol Ther* 2010; 31: 969.
21. Hoglen NC, Anselmo DM, Katori M, et al. A caspase inhibitor, IDN-6556, ameliorates early hepatic injury in an ex vivo rat model of warm and cold ischemia. *Liver Transpl* 2007; 13: 361.
22. Ueno Y, Ohmi T, Yamamoto M, et al. Orally-administered caspase inhibitor PF-03491390 is retained in the liver for prolonged periods with low systemic exposure, exerting a hepatoprotective effect against alpha-Fas–induced liver injury in a mouse model. *J Pharmacol Sci* 2007; 105: 201.
23. Canbay A, Feldstein A, Baskin-Bey E, et al. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *J Pharmacol Exp Ther* 2004; 308: 1191.
24. Emamaullee JA, Merani S, Toso C, et al. Porcine marginal mass islet autografts resist metabolic failure over time and are enhanced by early treatment with liraglutide. *Endocrinology* 2009; 150: 2145.
25. Ricordi C, Gray DW, Hering BJ, et al. Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 1990; 27: 185.
26. Barnett MJ, McGhee-Wilson D, Shapiro AM, et al. Variation in human islet viability based on different membrane integrity stains. *Cell Transplant* 2004; 13: 481.