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Evaluation of Viable β-cell Mass is Useful for Selecting Collagenase for Human Islet Isolation: Comparison of Collagenase NB1 and Liberase HI

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Abstract

Objectives—The selection of enzyme blend is critical for the success of human islet isolations. Liberase HI collagenase (Roche) has been introduced in the 1990's and widely used for clinical islet transplantation. More recently, a blend collagenase NB1 has been rendered available. The aim of this study was to evaluate the isolation outcomes and islet quality comparing human islet cells processed using NB1 and Liberase HI.

Methods—A total of 90 isolations processed using NB1 (n=40) or Liberase HI (n=50) was retrospectively analyzed. Islet yield, function *in vitro* and *in vivo*, cellular (including β-cell specific) viability and content, as well as isolation related factors were compared.

Results—No significant differences in donor related factors were found between the groups. There were also no significant differences in islet yields (NB1 vs. Liberase; 263,389±21,550 vs. 324,256±27,192 IEQ; P = n.s., respectively). The pancreata processed with NB1 showed a significantly longer digestion time (18.6±0.7 vs. 14.5±0.5 min, P <0.01), lower β-cell viability (54.3±3.4 vs. 72.0±2.1%, P < 0.01), β-cell mass (93,671±11,150 vs. 148,961± 12,812βIEQ, P<0.01) and viable β-cell mass (47,317±6,486 vs. 106,631±10,228 VβIEQ, P < 0.01) than Liberase HI. In addition, islets obtained with Liberase showed significantly better graft function in *in vivo* assessment of islet potency.

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Conclusions—The utilization of collagenase NB1 in human islet isolation was associated with significantly lower β-cell viability, mass and islet potency *in vivo* in our series when compared to Liberase HI even though there was no significant difference in islet yields between the groups. Evaluation of viable β-cell mass contained in human islet preparations will be useful for selecting enzyme blends.

Keywords

islet; transplantation; isolation; collagenase; cell viability; potency; nude mice

Introduction

Despite the widespread use of glucose monitoring and new insulin formulations, many individuals with diabetes still develop devastating secondary complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease (12, 21). The recent developments and improvements in islet transplantation related technology and immunosuppressant medications allow restoring near normal glucose control in patients with brittle type 1 diabetes with the minimal risk of serious hypoglycemic episodes that are associated with intensive insulin therapy (7, 13, 33, 41, 42).

The critical procedure of islet isolation is a mechanically-enhanced enzymatic digestion of the pancreas, which allows dissociation and freeing of the islets from the surrounding acinar tissue (27, 38). Collagenase plays a crucial role in dissociating the pancreas during enzymatic digestion phase. Liberase HI collagenase (Liberase; Boeringer-Mannheim/Roche) has been utilized as a standard blend for clinical islet transplantation since 1994 (28). More recently, the collagenase NB1 blend (Serva) has been rendered available and utilized for clinical trials in human islet isolation.

Incidentally, concerns on the potential risks of contamination of the animal tissues utilized in the manufacturing of Liberase HI led to the decision to voluntarily suspend its use for clinical-grade human islet cell products worldwide. Thus, the only alternative option for clinical use became collagenase NB1. This conversion created substantial effects on islet transplant activity through the significant fluctuation of islet yield and quality.

In this study, we retrospectively analyzed and compared human islet isolations processed using Liberase HI and collagenase NB1 blends in terms of isolation outcomes, isolation related factors and islet quality. Our β-cell-specific islet assessment methods revealed that significantly better quality of islets processed using Liberase HI, even though classic assessment methods such as FDA/PI could not distinguish.

Materials and Method

Human pancreata from multi-organ donors with consent for research and/or clinical use were processed deceased at the Human Islet Cell Processing Facility of the Diabetes Research Institute at the University of Miami Leonard M. Miller School of Medicine (DRI-UM). Fifty-seven human pancreata were processed between March 2007 and December 2009 using collagenase NB1 (9 lots, 2,580±162 Wunsch) with Neutral Protease NB (6 lots: 269±105 U/Vial) (SERVA Electrophoresis, Islandia, NY). Based on the donor selection criteria (Table 1), 17 out of 57 pancreata were excluded from this study. As control group, we used human pancreata that met the same criteria and were processed using Liberase HI (6 lots: 2,276±187 Wunsch) (Roche, Indianapolis, IN) between February 2004 and August 2007.

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Human islet isolation

All islet isolations were performed using a modified automated method with the same protocol, as described (38). Briefly, pancreata were immersed in an antibiotic solution prior to removing the surrounding vascular and adipose tissues. After cleaning, the pancreas was cut at the neck dividing the pancreas into two portions, the head and tail. Two 16-gauge catheters were then inserted into the main pancreatic duct of both pancreas portions. The dissociation buffer containing either collagenase NB1 or Liberase HI blend was perfused into the main pancreatic duct with a pump that automatically adjusts the flow rate to maintain constant pressure (38). After achievement of pancreas distension, 7–9 pieces were transferred into a digestion chamber with the same dissociation solution. The chamber was gently shaken to allow the pancreas to dissociate by both internal and external mechanical and enzymatic digestion. The digested pancreatic tissue was diluted, collected, and washed three times with chilled solutions. The islets were purified with either only continuous or continuous following discontinuous density gradients using a COBE 2991 Cell Processor (Gambro Laboratories, Denver, CO) at 4°C (17, 32). Islet yield and purity were determined by dithizone (DTZ) staining (37). Islet counts were obtained before (Pre-) and after (Post-) purification as well as after culture by scoring islets with incremental diameter size ranges and data expressed in islet particulate number and islet equivalent number (IEQ).

Fractional β-cell viability assay

Fractional β-cell viability was determined using flow cytometry, as reported (15,19,20). Single cell suspensions were incubated for 30 min at 37°C in phosphate-buffered saline (PBS) without $Ca2^{+}$ and $Mg2^{+}$ and with Newport Green PDX acetoxymethylether (NG; 1 mM) and tetramethylrhodamineethylester (TMRE; 100 ng/ml; both from Molecular Probes). Cells were stained with 7-aminoactinomycin D (7-AAD; Molecular Probes), a marker of cell death. Cell suspensions were analyzed (minimum 3.0×10^{4} events) using a FACScan cytometer with the CellQuest-pro software (Becton Dickinson, Mountain View, CA, USA). After counting, the dead cells (7-AAD^{-}) were excluded from further analysis, live β-cells (NG^{bright}) were analyzed for mitochondrial membrane potential (TMRE), which allows discriminating between viable ($TMRE^{+}$) and apoptotic ($TMRE^{-}$) cells. Apoptosis was evaluated selectively in the NG^{bright} cell population. The percentage of $TMRE^{+}$ on live β-cells (NG^{bright}) subset was determined based on the gate of TMRE unstained samples.

Cellular composition assay

The content of α-, β-, δ- and pancreatic polypeptide cells was determined by laser scanning cytometry (LSC/iCys; CompuCyte, Cambrige, MA), as described (15,16). Briefly, dispersed cells were fixed on glass slides with 2.5% paraformaldehyde (Electron Microscopy Sciences, Washington, PA). After incubation with Protein Block (BioGenex, San Ramon, CA) at room temperature (RT) for 30 min to reduce non-specific binding, cells were incubated at RT for 2 hr with the following primary antibodies: mouse monoclonal antibody to c-peptide (1:100 dilution, Abcam Inc., Cambridge, MA), mouse monoclonal antibody to glucagon (1:500; Sigma, St. Luis, MO), rabbit polyclonal antibody to somatostatin (1:500 dilution, Dako North America Inc., Carpinteria, CA), and rabbit polyclonal antibody to pancreatic polypeptide (PP; 1:1000 dilution, Dako). After washing in Optimax Wash Buffer (Bio-Genex, San Ramon, CA), cells were incubated at room temperature for 1 hr with Alexa Fluor 488 goat anti-mouse IgG (1:200 dilution, Molecular Probes, Eugene, OR), Alexa Fluor 647 goat anti-mouse IgG (1:200 dilution, Molecular Probes) antibodies and 4′,6-diamidino-2-phenylindole (DAPI)(17ul/ml, Molecular Probes). The percentage of α-, β-, δ- and pancreatic polypeptide cell was automatically calculated with LSC/iCys software.

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Calculation of β-cell content within islet and absolute β-cell mass

Based on the analysis of immunostaining for endocrine cell markers by LSC/iCys, each parameter was calculated using the formulas:

$$F1{:}\beta\text{–cell content within islet}=\beta\text{–cell\%}/(\alpha+\beta+\delta+\text{PP cell\%})\times 100\ (\%)$$

$$F2{:}\beta\text{–cell mass }(\beta\text{IEQ})=\beta\text{–cell content within islet }(\%)\times\text{Total IEQ}$$

$$F3{:}\text{Viable }\beta\text{–cell mass }(V\beta\text{IEQ})=\beta\text{–cell mass}\times\text{the percentage of TMRE}^{+}\text{on live }\beta\text{–cells }(\text{NG}^{\text{bright}})\ (\%)$$

Fluorescein diacetate-propidium iodide viability staining

After culture, 50–100 IEQ were transferred to 5-ml centrifuge tube and settled for 2–3 min. After removal of the supernatant, the settled islets were collected and resuspended in phosphate-buffered saline (PBS) and placed into a 35×10 mm Petri dish. The islets were stained, in the dark, with the 0.46 μM of fluorescein diacetate (FDA) and 14.34 μM propidium iodide (PI). Fifty individual and consecutively stained islets were assessed for percent viability by estimating the ratio of viable (green fluorescence) vs. nonviable (red fluorescence) cells using a fluorescent microscope, as described (30).

Glucose-stimulated insulin release

To determine the *in vitro* potency of islets, a static glucose challenge was performed as described (15). Briefly, after overnight culture, islets (50–100 IEQ) were incubated in parallel with either 2.8 or 20 mM glucose in culture medium for 2 h at 37°C. At the end of the incubation period, the supernatant was collected for insulin assessment by ELISA (Alpco, Salem, NH). A glucose stimulated insulin index was calculated by dividing the insulin content in the supernatant of the islet aliquots exposed to high glucose to that of the high glucose.

In vivo assessment of islet potency

Animal procedures were approved by the IACUC, and performed in the DRI's Preclinical Cell Processing and Translational Models Core. Athymic nu/nu (nude) mice (Harlan Laboratories, Indianapolis, IN) were housed in virus-antibody-free rooms in micro-isolated cages, having free access to autoclaved chow and water. Animals were rendered diabetic via intravenous administration of 200 mg/kg of Streptozotocin (Sigma, St. Luis, MO). Non-fasting blood glucose was assessed with a glucometer (OneTouch Ultra2; Life Scan, Milpitas, CA). Mice with sustained hyperglycemia (>300 mg/dL) were used as islet graft recipients. Islet grafts of 2000 IEQ each were implanted under the left kidney capsule, as described (15). Collectively, 41 mice received islet grafts from 33 human preparations processed using collagenase NB1 and 40 mice received islets from 26 human preparations isolated with Liberase enzyme blend. Average percentage of transplanted mice that reverted diabetes per human islet preparation and days required to reverse diabetes were assessed (17).

Statistical analysis

Data were analyzed using Excel (Windows) and Prism 4.0 (GraphPad) software. Data are shown as mean ± standard error of the means (s.e.m.). Statistical analyses were performed

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using methods appropriate to each specific analysis including Student's *t*-tests for two sample comparisons of independent groups and Bonferroni correction followed by ANOVA for six samples comparison. Statistical significance was considered for *P*-values < 0.05.

Results

Characteristics of donors

The donor characteristics in each group are shown in Table 2. There was no significant difference in age, gender ratio (male/female), body height, body weight, body mass index (BMI), cold ischemia time (CIT), and pancreas weight between the two groups.

Isolation-related variables

The islet isolation related variables in each group are summarized in Table 3. The digestion time in the collagenase NB1 group was significantly longer, when compared with the Liberase group (18.6±0.7 *vs.* 14.5±0.5 minutes P<0.01). There was no significant difference in the undigested pancreas weight and in the post-purification islet yields between the two groups. There was also no significant difference between the groups in terms of the transplant rate and successful isolation rate (post-purification islet yield: more than 250,000IEQ) (Liberase *vs.* NB1: 54.5% *vs.* 36.8%, P=N.S., 52.9% *vs.* 41.2%, P=N.S., respectively). However, there were statistically significant differences between the two groups in the post and pre-purification islet particle number (Pre-purification: 405,965±21,910 *vs.* 315,178±27,609, P<0.05; Post-purification: 270,986±19,870 *vs.* 188,731±19,732, P<0.05, in Liberase and NB1, respectively).

The average size of the islets (IEQ/IPN) pre- and post-purification in the Liberase group was significantly smaller than those in NB1 group (Pre-purification: 1.13± 0.07 *vs.* 1.31±0.10, P=0.053; Post-purification: 1.28±0.10 *vs.* 1.53 ± 0.10, P<0.05, in Liberase and NB1, respectively), even though the NB1 group had significantly longer digestion times. The results suggested that enzymatic activity of NB1 might be lower when compared to that of Liberase. However, there were no significant differences between the two groups in the percentage of embedded islets recovered (Liberase vs. NB1: 13.9±1.9 *vs.* 13.8±2.5, P=N.S.).

Islet Quality

Viability—To examine the effects of the two different collagenase blends on islet quality, islet preparations were assessed using FDA/PI, which has been currently used for clinical trials. There was no significant difference in islet cell viability assessed by FDA/PI between the two groups (Liberase *vs.* NB1: 87.7±1.1% *vs.* 90.7±2.1%, P=N.S.). However, fractional β-cell viability, which allows evaluating apoptotic cells as well as dead cells by flow cytometry (Table 4), showed that islets in the Liberase group had significantly higher β-cell viability (the percentage of $TMRE^+$) when compared to the NB1 group (Liberase vs. NB1: 72.0±2.0% *vs.* 54.5±3.4 %, P<0.05) although both groups had similar percentages of dead cells ($7AAD^+$).

β-cell content and islet mass—To assess the effects of the two enzyme blends on β-cell content/mass contained in each human islet preparation, we obtained single islet cell suspensions and stained them for pancreatic hormones (Table 4). The β-cell content and β-cell mass (βIEQ) for each islet preparation were calculated. The Liberase group showed significantly higher β-cell content within islet than the NB1 group (46.3±1.6% *vs.* 33.4±2.0%; P<0.01). The β-cell mass in the Liberase group was also significantly higher than in the NB1 group (148,451±12,812 *vs.* 91,351±11,150 βIEQ, P<0.01) even though there was no significant difference in total islet yield between the two groups. Moreover, there was significant difference between the groups in terms of the viable β-cell mass

(VβIEQ), which was most associated with *in vivo* islet potency (Liberase *vs.* NB1: 106,274±10,228 *vs.* 47,317±6,486 VβIEQ, P<0.01) (Figure 1A–C).

Islet potency

In vitro: glucose stimulation insulin index—

The glucose stimulation insulin index in the NB1 group was significantly lower than in the Liberase group (1.65±0.22 *vs.* 2.37±0.21, P<0.01) (Table 4).

In vivo: islet potency in the diabetic nude mouse model—

The average reversal rate of diabetes in transplanted mice in the Liberase and NB1 groups were 81% and 69%, respectively (P=N.S., Figure 2A). Although there was no significant difference in the reversal rate, it took significantly more days to reverse diabetes in the NB1 group than in the Liberase group (P<0.05; Figure 2B).

Discussion

In this study, we have compared the effects of two clinical grade collagenase blends, Liberase and NB1, on islet yield and β-cell quality in human islet isolations. Although islet yields assessed by DTZ staining were comparable between the two groups, β-cell mass assessed by C-peptide staining in the Liberase group was significantly higher. Assessment of islet cell viability by FDA/PI, which has been the gold standard method used for ongoing clinical trials, did not detect any differences in the viability of islets between the two groups. However, the Liberase group showed significantly higher β-cell specific viability than the NB1 group when viability was assessed by FACS, which allows evaluating both dead and apoptotic cells (15). Moreover, islets in the Liberase group demonstrated significantly better quality both *in vitro* and *in vivo* islet potency. Our results consistently indicated that the quality of islets processed using the collagenase NB1 blend was not as good as that of islets processed using Liberase HI, even through islets yields were comparable. Evaluation of viable β-cell mass contained in human islet preparations will be of assistance in for selecting enzyme blends.

Since *C. histolyticum* collagenase became commercially available in the early 1960s, it has been widely used as a tissue-dispersing enzyme (14, 24, 34). A variety of collagenases/proteases produced by this technology allows for degrading various types of collagen and gelatin. Therefore, enzyme derived from *C. histolyticum* is called “crude collagenase” (11). The Liberase HI was introduced in November 1994 as the first dissociation enzyme blend developed to isolate human islets (26, 28, 36). This product contained purified collagenase (both class I and II) from *C. histolyticum* and a purified bacterial neutral protease (thermolysin or Dispase®)(28). The development of Liberase HI has dramatically changed human islet isolation (28), contributing to obtaining reproducibly high numbers of islets from large animal pancreata, including human, and establishing human islet transplantation as an option of therapies for patients with type 1 diabetes (41). This has been attributed in part to the activity and purity of the enzyme blend, as well as to its low endotoxin contamination (5). Thus, Liberase HI had been used for clinical islet isolation until it was discontinued due to the potential, albeit low, risk of prion disease transmission. Since then, collagenase NB1 has been utilized as the only enzyme blend available for clinical purposes.

Several groups have reported the comparison between Liberase HI and collagenase NB1 blends in terms of the efficiency of islet isolations yield and islet quality (2, 8, 9, 10, 39). Recently Brandhorst *et al.* reported that collagenase NB1 is less efficient for pancreas dissociation than Liberase HI, however, describing that it may be less harmful to pancreatic tissues (8), and that the glucose-stimulated insulin release index and insulin content in the were significantly higher than in the Liberase group. In our study, we did not observe

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significant difference in terms of islet yields from human pancreata. In agreement with the previous report, we observed that the both β-cell mass and the VβIEQ in the NB1 group were significantly lower than in the Liberase group. Furthermore, both *in vitro* and *in vivo* islet function in the NB1 groups were significantly lower than in the Liberase group.

One possible explanation in the discrepancy between their report and our data may be related to lot-to-lot variability in the NB1 collagenase blend. In particular, a total of nine different lots of NB1 blend were used in our study and among them, five were utilized more than four times. Although this data was not conclusive due to the small number of samples, there was significant differences observed in islet yield and β-cell mass (islet yield, A: 491,625 +/− 70,906, B: 269,765 +/− 56,087, C: 257,603 +/− 6,048, D: 259,622 +/− 38,262 and E: 241,012 +/− 69,536 IEQ, ANOVA, P = 0.055); β-cell mass, A : 193,913+/−32,620, B : 49,349+/−13,504,C : 75,779+/−25,749, D : 103,953+/−22,491 and E : 101,659+/− 37,073 βIEQ, ANOVA P < 0.01: Bonferroni correction A vs. C, D;P < 0.05). In fact, the highest β-cell mass in lot A was comparable to the one in the Liberase group (N.S.). As others reported (2), our data also showed significantly longer digestion times in the NB1 group. The longer exposure of pancreatic tissues to active enzyme at high temperature (37°C) results in the lower islet viability through the enzymatic toxicity and hypoxia (29, 40). Even though NB1 blend might be less harmful to islet cells, some of the lots tested showed less potency in terms of pancreas dissociation, which exposed the tissue to longer to a detrimental environment. Notably, Libease HI has also been reported to have lot-to-lot variability (4, 35, 43). A large-scale, multi-center study may be needed to assess the impact of lot-to-lot variability of the NB1 blend.

Anazawa *et al.* recently reported the comparison between Liberase HI and NB1 blends utilized for clinical auto-islet transplantation for patients with chronic pancreatitis (2). They demonstrated that total islet yields with no purification in both groups were similar, and that the percentage of embedded islets was significantly higher in the NB1 group compared to the Liberase group. The purification procedure is not always necessary for auto-islet transplantation unless the volume of whole islet preparation is high, which may increase the risk of intrahepatic thrombo-embolic events (31). However, in allogeneic islet transplantation, the purification process is a key for successful islet isolation and to reduce the volume of antigenic tissue implanted into the recipients. Even though the purification protocols have been improved in recent years, it remains still difficult to separate embedded islets from exocrine tissue when compared to non-embedded islets (3, 17, 32). This phenomenon might have contributed to the lower transplant rate registered in the islet isolation processed using NB1 blend. In human islet isolation, purifying islet from other pancreatic tissue had been challenging until the large-scale purification method using COBE 2991 cell processor was reported (1, 25). The selection of the purification method or medium might be a possible explanation of the discrepancy among islet processing centers.

Neutral proteases have been recently reported to play an important role in enzymatic digestion (6, 7, 22, 23). Kin *et al.* reported that the optimization of the neutral protease dosage based on pancreas weight rather than the on class II/class I collagenase activity ratio was more crucial in successful islet isolation (23). Furthermore, they developed a pancreas digestion protocol optimized for NB1 enzyme, where only collagenase was injected into the pancreas through the duct, and then neutral protease was added to the circulating system during the digestion phase (22). Liberase HI and NB1 blends have different type of neutral proteases. Little data has been available to date in regards to the effects of neutral proteases on human islet quality and isolation yields. Although the neutral protease will be useful for shortening the digestion time through helping the dissociation of pancreatic tissues, it may be toxic for islet cells. However, it may be beneficial for islet cells through the degradation of active collagenase I/II. Further study addressing these issues will be needed.

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In conclusion, the present study demonstrated that the utilization of collagenase NB1 blend was associated with significantly decreased the β-cell mass, viability, and function when compared to Liberase, even though islet yield was comparable. Collagenese NB1 might be less potent than Liberase in terms of the disintegration of the pancreas, which caused a longer digestion time and eventually exposed islet cells in harsh condition for a long time. Further understanding of the lot-to-lot variability, of the role of neutral proteases and thermolysin, as well as the optimization and/or selection of isolation procedures based on the type of the utilized enzyme may be of assistance in improving islet isolation outcomes, leading to increased successful islet transplantation.

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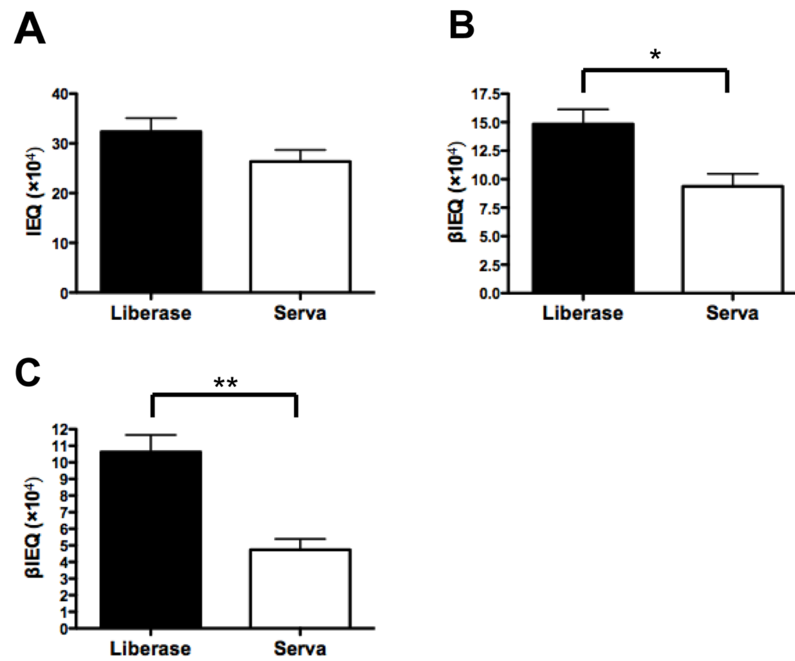

Figure 1. Isolation outcomes

A: There was no significant difference in post-purification islet yields between the two groups (Collagenase NB1 vs. Liberase; 263,389 ± 21550 vs. 323,616 ± 27,192 IEQ; n.s.). **B, C:** The NB1 group showed significantly lower β-cell mass (93,671 ± 11,150 vs. 148,451 ± 12,812 βIEQ, * $P < 0.05$) and viable β-cell mass (47,317 ± 6,486 vs. 106,274 ± 10,228 VβIEQ, ** $P < 0.01$).

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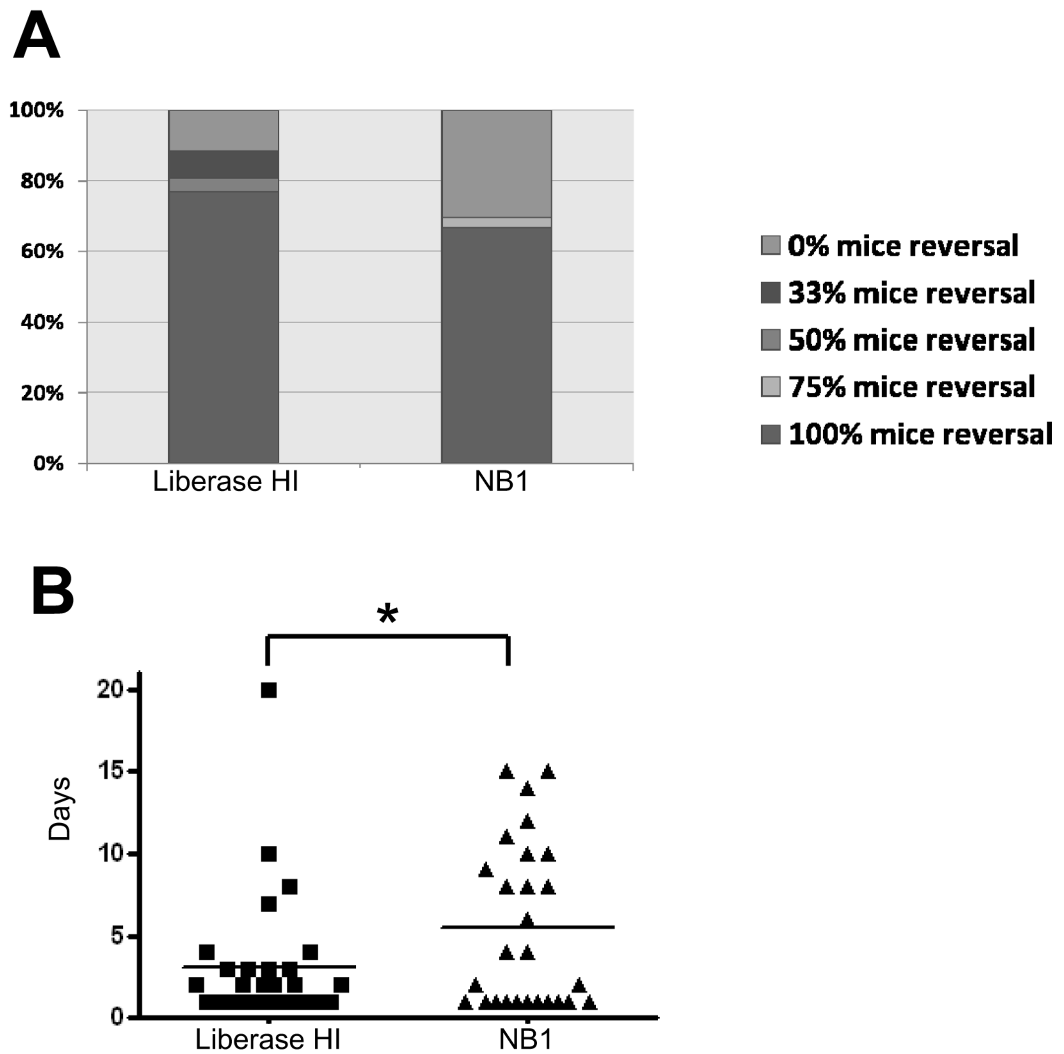

Figure 2. In vivo potency of human islet preparations

A, B: Average percentage of transplanted mice that reversed diabetes per human islet preparation processed using collagenase NB1 or Liberase HI. The reversal rate in diabetic nude mice transplanted with human islet preparations showed no significant differences (A). The time required for the reversal of diabetes between the experimental groups was significantly longer in the NB1 group than in the Liberase group (B) ($P < 0.05$).

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Table 1

Exclusion criteria

Exclusion Criteria
Experimental isolation
Type 1 or 2 Diabetes donor
Perfusion using warm collgenase solution
Pregnant donor
Used for auto-islet transplantation
Septicemia

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Table 2

Pancreas donor variables

	Liberase	NB1	P (S *vs*. L)
N	50	40	
Age (years)	43.8 ± 1.4	40.0 ± 1.7	N.S.
Gender (Male:Female)	35 :15	26 : 14	N.S.
Body height (cm)	172.1 ± 1.2	170.5 ± 1.6	N.S.
Body weight (kg)	89.1 ± 2.6	84.9 ± 2.7	N.S.
Body mass index (kg/m^2)	29.9 ± 0.7	28.5 ± 0.7	N.S.
Cold ischemic time (minutes)	617.6 ± 33.4	638.3 ± 39.1	N.S.
Pancreas weight (g)	106.2 ± 4.2	98.3 ± 3.8	N.S.

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Table 3

Isolation variables

	Liberase	Serva	P (L vs S)
Digestion time (minutes)	14.5 ± 0.5	18.57 ± 0.67	**< 0.01**
Embedded islet (%)	13.7 ± 2.4	13.9 ± 1.9	N.S.
Prepurification (IEQ)	434243 ± 27393	375751 ± 27487	N.S.
Prepurification (PN)	405929 ± 21910	315178 ± 27609	**< 0.05**
Postpurification (IEQ)	324256 ± 27192	263389 ± 21550	N.S.
Postpurification (PN)	270986 ± 19870	188731 ± 19732	**< 0.05**
Percent recovery (%)	73.8 ± 5.1	70.5 ± 5.1	N.S.
Undigested pancreas (g)	29.2 ± 2.5	25.2 ± 1.7	N.S.
IEQ/g	5931.5 ± 398.4	5672.8 ± 516.0	N.S.

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Table 4

Islet cell product assessment

	Liberase	NB1	P (L *vs.* S)
FDA/PI Viability (%)	90.7 ± 2.1 (n = 48)	87.7 ± 1.1 (n = 38)	N.S.
Static Incubation (%)	2.37 ± 0.21 (n = 45)	1.65 ± 0.22 (n = 36)	**< 0.01**
FACS analysis			
7AAD negative cells			
Living cells (%)	84.9 ± 1.1	74.7± 1.7	**< 0.01**
Beta cell viability (%)	72.0 ± 2.1	54.2 ± 3.3	**< 0.01**
Immunohistochemistry			
Beta cell (%)	22.2 ± 1.5	15.5 ± 1.7	**< 0.01**
Alpha cell (%)	17.4 ± 1.0	19.9 ± 1.5	N.S.
Delta cell (%)	4.4 ± 0.3	4.7 ± 0.4	N.S.
PP cell (%)	2.8 ± 0.3	2.0 ± 0.2	**< 0.05**
Purity (%)	46.1 ± 2.3	42.7 ± 2.9	N.S.
Beta cell content in islet	46.4 ± 1.6	33.5 ± 2.0	**< 0.01**