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# Pancreas Preservation with a Neutrophil Elastase Inhibitor, Alvelestat, Contributes to Improvement of Porcine Islet Isolation and Transplantation

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**Abstract:** For pancreatic islet transplantation, pancreas procurement, preservation, and islet isolation destroy cellular and non-cellular components and activate components such as resident neutrophils, which play an important role in the impairment of islet survival. It has been reported that inhibitors of neutrophil elastase (NE), such as sivelestat and  $\alpha$ 1-antitrypsin, could contribute to improvement of islet isolation and transplantation. In this study, we investigated whether pancreatic preservation with alvelestat, a novel NE inhibitor, improves porcine islet yield and function. Porcine pancreata were preserved with or without 5  $\mu$ M alvelestat for 18 h, and islet isolation was performed. The islet yields before and after purification were significantly higher in the alvelestat (+) group than in the alvelestat (−) group. After islet transplantation into streptozotocin-induced diabetic mice, blood glucose levels reached the normoglycemic range in 55% and 5% of diabetic mice in the alvelestat (+) and alvelestat (−) groups, respectively. These results suggest that pancreas preservation with alvelestat improves islet yield and graft function and could thus serve as a novel clinical strategy for improving the outcome of islet transplantation.

**Keywords:** pancreas preservation; alvelestat (AZD9668; MPH966); neutrophil elastase (NE); islet transplantation

## 1. Introduction

Pancreatic islet transplantation is a treatment option for patients with type 1 diabetes mellitus [1–5]. Islet transplantation is a minimally invasive approach to  $\beta$ -cell replacement compared with pancreas transplantation. However, the number of islets from one donor pancreas is usually insufficient to achieve insulin independence [1,6–8]. The procedure of islet isolation itself destroys cellular and non-cellular components of the pancreas and activation of some components, including resident neutrophils, macrophages, and T cells, which probably play an important role in the impairment of islet survival [6–10].

Some studies have shown that inhibitors of neutrophil elastase (NE), such as sivelestat and  $\alpha$ 1-antitrypsin, could contribute to the improvement of islet isolation and transplantation [11–14]. NE is a serine protease stored in azurophilic granules of neutrophils in its inactive form [15–17]. When NE is released after neutrophil exposure to inflammatory stimuli, it is fully active. Excessive release of NE degrades several extracellular matrix

components, such as elastin, laminins, and collagens, resulting in subsequent tissue damage [15–19]. NE activity reportedly increases during islet isolation, especially at the end of warm digestion by collagenase [11]. Supplementation of preservation solutions with sivelestat, a serine protease inhibitor, significantly improves islet yield and viability. Furthermore, treatment with sivelestat prolongs islet allograft survival in recipient mice [11]. In rats with hyperketonaemia, treatment with sivelestat sodium reduces serum cytokines and improves islet yields and functions in vitro [12].  $\alpha$ 1-Antitrypsin is also a serine protease inhibitor that inhibits NE [20–23], prolongs islet graft survival [13], and reduces expression of proinflammatory mediators [14] in mice by binding to gp96 (a heat-shock protein) [24].

Alvelestat (AZD9668, MPH966) is another NE inhibitor that is reversible and highly selective in biochemical and cellular assays. The inhibitory effect on NE induced by alvelestat is higher than that induced by sivelestat. It has been reported that alvelestat is effective in in vivo mouse and rat models of lung inflammation and degradation [25]. In rats, alvelestat reduces the diameters of abdominal aortic aneurysms (AAA) induced by injection of *Porphyromonas gingivalis* and limits the formation of calcium phosphate precipitation in AAA [26]. In mice with acute lung injury or acute respiratory distress syndrome, alvelestat exhibits potent cytoprotective and anti-inflammatory properties in human alveolar and bronchial epithelial cells by targeting neutrophil extracellular traps, improves survival rates, and reduces lung inflammation [27]. In clinical trials, alvelestat has been shown to reduce sputum inflammatory biomarkers in cystic fibrosis [28] and bronchiectasis [29]. In this study, we investigate whether the addition of alvelestat to preservation solution improves porcine islet yield and function.

## 2. Materials and Methods

### 2.1. Inhibitory Effects of Alvelestat against the Cytotoxicity of NE

Isolated porcine islets from pancreas preservation solution without alvelestat were cultured with Connaught Medical Research Laboratories 1066 medium (CMRL 1066; Sigma-Aldrich Japan, Tokyo, Japan) supplemented with 0.5% human serum albumin (HSA; Sigma-Aldrich Japan) with or without 1  $\mu$ g/mL NE (Sigma-Aldrich Japan) and 0–25  $\mu$ M alvelestat (AduoQ Bioscience, Irvine, CA, USA) for 24 h at 37 °C in 5% CO<sub>2</sub>. The islets were evaluated by double fluorescein diacetate/propidium iodide (FDA/PI; Sigma-Aldrich Japan) staining as described previously [30–33].

### 2.2. Pancreas Preservation Solution

A commercially available extracellular-type trehalose-containing Kyoto (ETK) solution (Otsuka Pharmaceutical Factory, Naruto, Japan) supplemented with 5  $\mu$ M alvelestat was used as preservation solution. Dimethyl sulfoxide (DMSO; Sigma-Aldrich Japan) plus phosphate-buffered saline (PBS; Sigma-Aldrich Japan) was used to dissolve alvelestat (alvelestat(+)), and DMSO plus PBS without alvelestat (alvelestat(–)) was used as the vehicle control.

### 2.3. Procurement, Preservation, and Islet Isolation of Porcine Pancreata

We obtained 20 porcine pancreata (female; three years) from a local slaughterhouse. The operation was started approximately 10 min after the animal's heartbeat stopped. All pancreata were removed using a standardised technique to minimise warm ischemic time (WIT). We inserted a catheter into the main pancreatic duct and infused preservation solution through the intraductal cannula (1 mL/g of pancreas) [34,35]. The pancreata were then stored in preservation solution at 4 °C for approximately 18 h. The “operation time” was defined as the time from the start of the operation until the removal of the pancreas. The WIT was defined as the time from the cessation of the animal's heartbeat until placement of the pancreas into the preservation solution. The cold ischemic time, phase I period, and phase II period were defined as described previously [5,35].

To isolate the islets, the ducts were perfused in a controlled fashion with a cold enzyme blend of Liberase T-Flex (1.0 mg/mL) with thermolysin (0.075 mg/mL) (Roche Diagnostics

Corporation, Indianapolis, IN, USA). The islets were then separated by gentle mechanical dissociation [5,35–39] and purified using a continuous gradient of purification solution [40]. To generate purification solutions, iodixanol was combined with preservation solution. We adopted bottle purification (size 500 mL; Nalgene, Rochester, NY, USA) in this step [35,40]. The digested tissue was divided in half so that equal amounts of tissue were used for each bottle. A gradient was generated using a gradient marker (Biorep Technologies, Miami Lakes, FL, USA), and candy-cane-shaped stainless steel pipes (length, 30 cm; UMIHIRA, Kyoto, Japan) to enable loading from the low-density solution to high-density solution, leaving the stainless steel pipe in place. After generating a continuous gradient, the digested tissue was loaded as the top layer [40]. The bottles were centrifuged at 1000 rpm ( $235\times g$ ) for 5 min at 4 °C. After centrifugation, about 9 fractions (50 mL each) were collected and examined for purity.

#### 2.4. Assessment of Islet Function

Dithizone (DTZ; Sigma-Aldrich) staining, scoring of gross morphology (score), and double FDA/PI staining were performed as described previously [1,30–33,35]. The crude number of islets in each diameter class was determined by counting islets after DTZ staining using an optical graticule. The crude number of islets was then converted to the standard number of islet equivalents (IE; diameter standardized to 150  $\mu\text{m}$ ) [1,30–33,35]. Islet function was assessed by monitoring the insulin secretory response of the purified islets during glucose stimulation using the procedure described previously [1,2,35,41–43]. The data were expressed as the mean  $\pm$  standard error of the mean (SE).

#### 2.5. Measurement of ATP Production

To measure the production of ATP, isolated islets in each group were cultured overnight with CMRL-1066 medium supplemented with 0.5% HSA, washed twice with ice-cold PBS, and solubilized. The amount of ATP was measured using an ATP assay system (Toyo Inki, Tokyo, Japan) according to the manufacturer's instructions. Briefly, after allowing the reagents to equilibrate to room temperature, 10  $\mu\text{L}$  of cell extract was added to 100  $\mu\text{L}$  of reagent. The samples were measured using a luminometer [35].

#### 2.6. In Vivo Assessment

For in vivo assessment of the islet function, nude mice (six-week-old, male; Charles River Laboratories Japan, Inc., Kanagawa, Japan) rendered diabetic by a single injection of streptozotocin (STZ) at a dose of 120 mg/kg were used. When the non-fasting blood glucose level exceeded 350 mg/dL for 2 consecutive days, the mice were considered to be diabetic. Marginal numbered porcine islets (1500 IEs) obtained from each group were transplanted into the renal subcapsular space of the left kidney of an immunodeficient diabetic mouse immediately after isolation [33,34,39,41]. Normoglycemia was defined as two consecutive blood glucose level measurements of less than 200 mg/dL. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus.

#### 2.7. Statistical Analysis

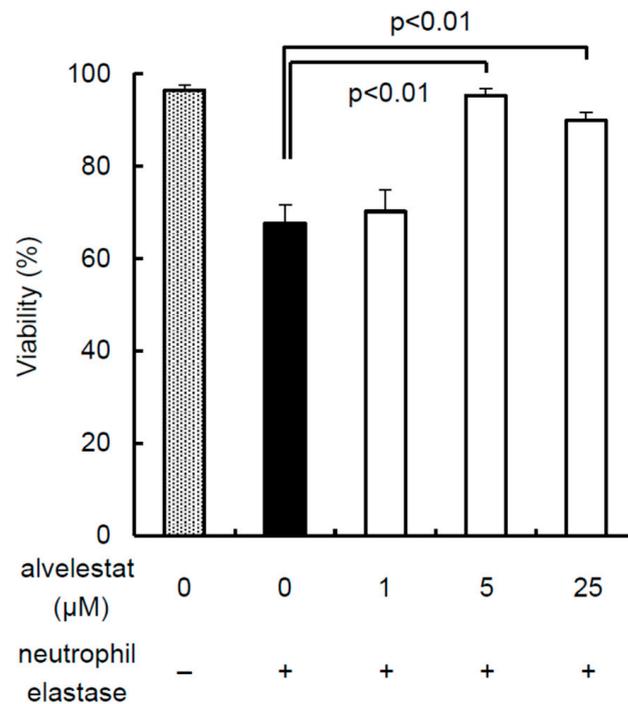
All data are expressed as mean  $\pm$  standard error. Student's *t*-test was performed to compare two samples from independent groups using Microsoft Excel. The differences in the duration of graft survival between the groups were evaluated using the Kaplan–Meier log-rank test, which was performed using StatView software. Statistical significance was set at  $p < 0.05$ .

### 3. Results

#### 3.1. Inhibitory Effects of Alvelestat against the Cytotoxicity of NE

To evaluate the inhibitory effect of alvelestat against the cytotoxicity of NE, isolated porcine islets from pancreas preservation solution without alvelestat were cultured with or

without 1  $\mu\text{g}/\text{mL}$  NE and 0–25  $\mu\text{M}$  alvelestat for 24 h. Islet viability was decreased as a result of treatment with NE and was recovered by treatment with 5 and 25  $\mu\text{M}$  alvelestat but not by treatment with 1  $\mu\text{M}$  alvelestat (Figure 1). The following experiments were performed using 5  $\mu\text{M}$  alvelestat.



**Figure 1.** Inhibitory effect of alvelestat against the cytotoxicity of neutrophil elastase. Isolated porcine islets were cultured with or without 1  $\mu\text{g}/\text{mL}$  neutrophil elastase and 0–25  $\mu\text{M}$  alvelestat for 24 h. The islets were evaluated using double fluorescein diacetate/propidium iodide (FDA/PI) staining.

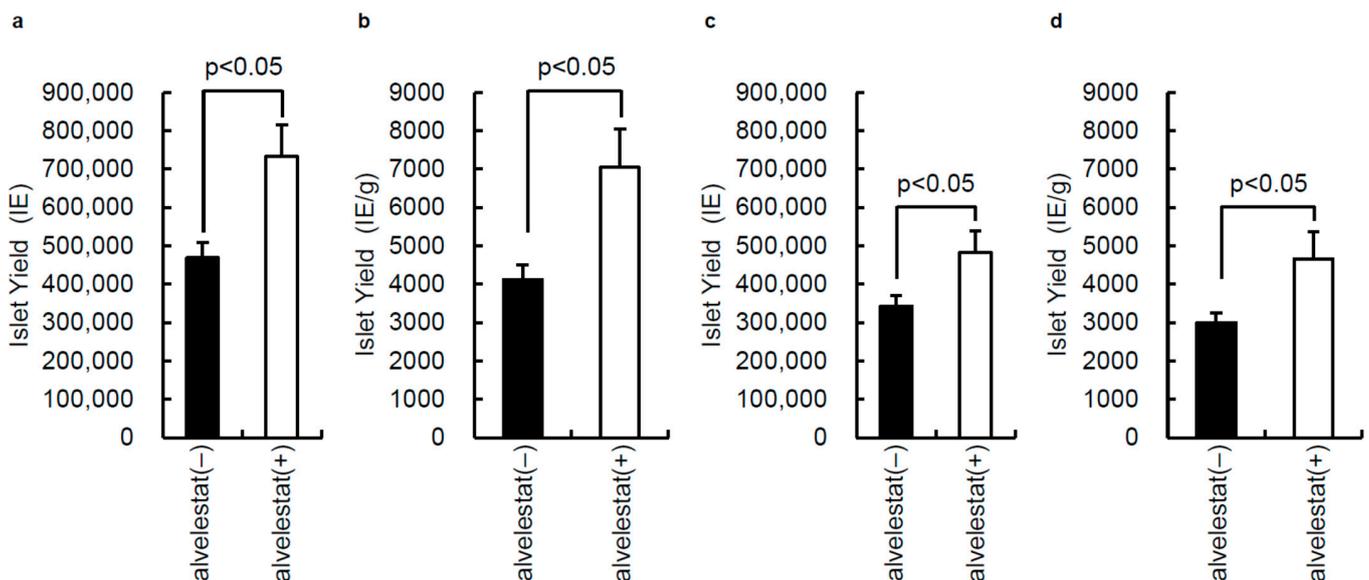
### 3.2. Effect of Alvelestat on Porcine Islet Isolation

To assess the effect of alvelestat on islet isolation, we performed 20 porcine islet isolations from 21 November 2020 to 6 November 2021. Porcine pancreata were preserved with or without 5  $\mu\text{M}$  alvelestat for approximately 18 h, and islet isolations were performed. The characteristics of porcine pancreata and islets before purification are summarized in Table 1. There were no significant differences in pancreas size, the operation time (the time elapsed between the start of the operation and removal of the pancreas), warm ischemic time (the time elapsed between the removal of the animal's blood and placement of the pancreas into the preservation solution), cold ischemic time (the time elapsed between the placement of the pancreas into the preservation solution and the start of islet isolation), phase I (digestion time), or phase II (collection time) between the two groups. In contrast, the islet yield before purification of the alvelestat (+) group ( $n = 10$ ) was significantly higher than that of the alvelestat (−) group ( $n = 10$ ) ( $p < 0.05$ ; Figure 2a,b).

**Table 1.** Characteristics of the pancreatic tissue and isolated islets in the alvelestat (−) group and the alvelestat (+) group.

Characteristic	Alvelestat (−) (n = 10)	Alvelestat (+) (n = 10)
Pancreas size (g)	120.1 ± 8.9	107.9 ± 5.6
Operation time (min)	4.9 ± 0.4	5.2 ± 0.2
Warm ischemic time (min)	27.1 ± 1.2	27.2 ± 0.8
Cold ischemic time (min)	1116.3 ± 9.4	1087.0 ± 12.7
Phase I period (min)	11.1 ± 0.5	11.6 ± 0.5
Phase II period (min)	38.9 ± 0.8	40.5 ± 0.7
Embedded islets (%)	12.0 ± 1.9	12.0 ± 1.3
Viability (%)	96.7 ± 0.5	96.5 ± 0.6
Purity (%)	64.2 ± 2.4	59.8 ± 3.7
Post-purification recovery (%) <sup>1</sup>	76.3 ± 3.2	67.4 ± 3.1
Score	9.5 ± 0.2	9.7 ± 0.1

The data are expressed as the mean ± SE. <sup>1</sup> Post-purification recovery (%) = IE after purification/IE before purification × 100.



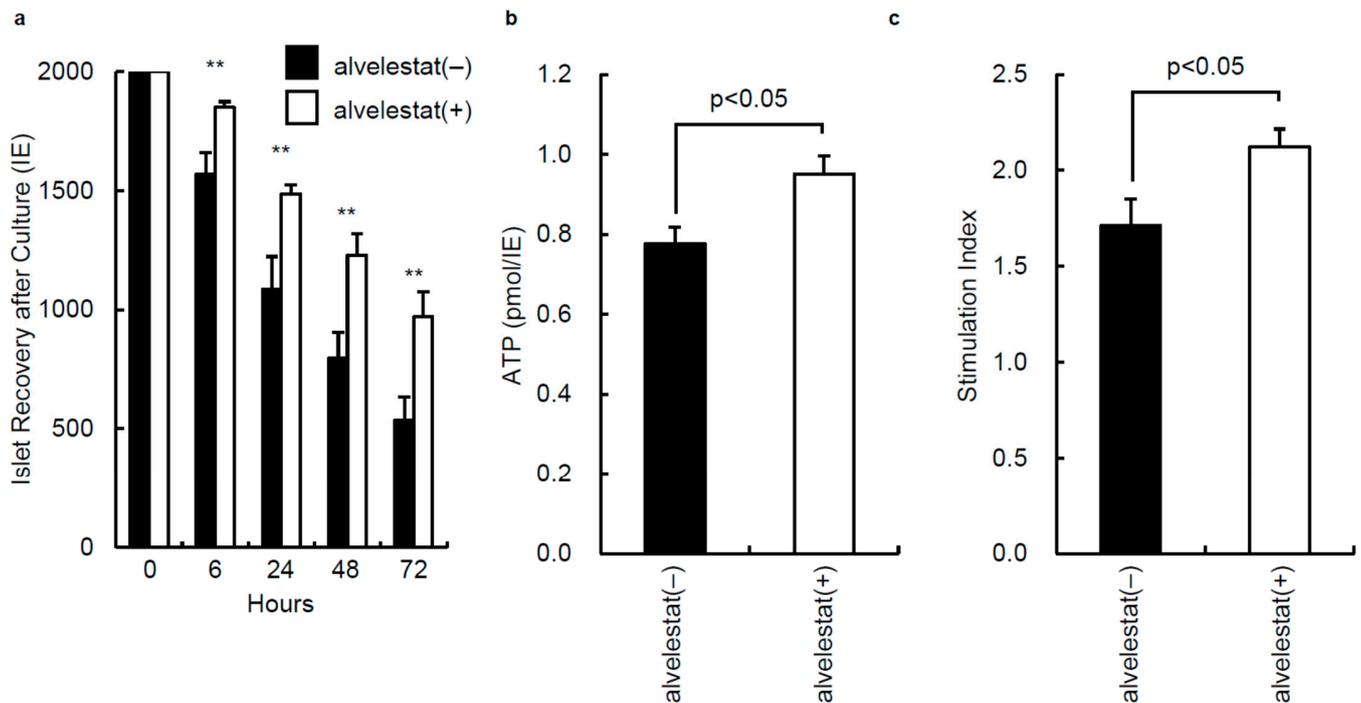
**Figure 2.** Effect of alvelestat on islet isolation. Porcine pancreata were preserved with or without 5  $\mu$ M alvelestat for approximately 18 h, and islet isolation was performed. (a) Islet yield before purification. (b) Islet yield per gram of pancreas before purification. (c) Islet yield after purification. (d) Islet yield per gram of pancreas after purification. Alvelestat (−) group:  $n = 10$ ; alvelestat (+) group:  $n = 10$ . Data are expressed as the mean ± SE.

The islet characteristics after purification are presented in Table 1. The yield of islets after islet purification of the alvelestat (+) group was significantly higher than that of the alvelestat (−) group ( $p < 0.05$ ; Figure 2c,d). No other characteristics were significantly different between the two groups.

### 3.3. In Vitro Assessment of Isolated Islets from Porcine Pancreata Preserved with or without Alvelestat

To investigate whether the addition of alvelestat to preservation solution can prevent the reduction in the number of islets during culturing, the IE was compared between the two groups after culturing for 6, 24, 48, and 72 h. The IE in the alvelestat (−) group ( $n = 3$ ) were significantly lower than the alvelestat (+) group ( $n = 3$ ) (Figure 3a). These data show that the addition of alvelestat to the preservation solution prevents a reduction in the number of islets during culture.

To evaluate islet function *in vitro*, the ATP content in each group was measured. The ATP content in the alvelestat (–) group ( $n = 10$ ) was significantly lower than that in the alvelestat (+) group ( $n = 10$ ) (Figure 3b). The stimulation index of isolated islets was also measured. The insulin production according to high glucose and stimulation index values in the alvelestat (+) group ( $n = 10$ ) were significantly higher than those in the alvelestat (–) group ( $n = 10$ ) (Figure 3c).



**Figure 3.** *In vitro* effect of alvelestat on isolated porcine islet function. (a) Number of islets after culturing. Immediately after islet isolation, 2000 IE from each group ( $n = 3$  each) were cultured for 72 h. The islets were counted to calculate the IE for each group after 6, 24, 48, and 72 h of culturing. \*\*  $p < 0.01$ . (b) The adenosine triphosphate (ATP) content in each group was measured using an ATP assay system. Alvelestat (–) group:  $n = 10$ ; alvelestat (+) group:  $n = 10$ . (c) The stimulation index was measured in each group. Alvelestat (–) group:  $n = 10$ ; alvelestat (+) group:  $n = 10$ . Data are expressed as the mean  $\pm$  SE.

### 3.4. *In Vivo* Assessment of Porcine Islets from Alvelestat (+) and Alvelestat (–) Groups

To assess the islet graft function *in vivo*, marginal numbered porcine islets (1500 IEs) from each group ( $n = 20$  from 10 islet isolations,  $n = 2$  per isolation) were transplanted immediately after isolation under the kidney capsule of STZ-induced diabetic nude mice. The blood glucose levels in 11 of 20 mice (55%) from the alvelestat (+) group decreased and reached normoglycaemia. In contrast, 19 of 20 mice (95%) from the alvelestat (–) group were hyperglycaemic (Table 2). Thus, the number of mice that became normoglycemic after islet transplantation differed significantly between the alvelestat (+) and alvelestat (–) groups ( $p < 0.01$ ). These results revealed that pancreatic preservation with alvelestat improved islet graft function.

**Table 2.** In vivo functional assay.

	Alvelestat (–)	Alvelestat (+)
No. of Transplanted Mice	20	20
No. of Cured Mice	1	11
%	5	55
<i>p</i> -Value vs. alvelestat (–)		<0.01

Marginal numbered porcine islets (1500 IEs) from each group were transplanted immediately after isolation into immunodeficient diabetic mice. Normoglycaemia was defined as <200 mg/dL under non-fasting conditions for more than 2 consecutive days.

#### 4. Discussion

In this study, we showed that the addition of alvelestat to the preservation solution increased islet yield and improved the function of isolated islets both in vitro and in vivo. These findings imply that alvelestat has protective effects on the preservation of the pancreas and islets. Donor pancreata are damaged by hypotension, tissue ischemia due to peripheral vasoconstriction, and release of stress hormones and inflammatory cytokines [44–47]. Donor pancreata are then exposed to warm/cold ischemia during procurement and organ preservation [48,49]. Furthermore, islet isolation, which includes warm digestion, tissue shaking, and cold purification/washing with hypoxia, causes cell damage [44]. It has been reported that NE activity increases during islet isolation, especially at the end of warm digestion by collagenase, with cytotoxic effects against isolated islets [11]. NE injures the membrane of macrophages, acinar cells, and islets [11]. NE also causes the activation of macrophages and acinar cells, leading to proinflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$  [50]. Therefore, alvelestat, sivelestat, and  $\alpha$ 1-antitrypsin can improve the outcome of islet isolation and transplantation.

ETK solution was used as the preservation solution in this study. We previously reported that a modified ETK solution containing a trypsin inhibitor (ulinstatin) significantly improved the islet yield compared with preservation in the University of Wisconsin (UW) solution for islet isolation [39,51]. ETK solution is advantageous for islet transplantation because the inhibitory effect of collagenase activity by ETK solution is weaker than that of UW solution [39]. Moreover, ETK solution has a high-sodium/low-potassium composition, whereas UW solution has a low-sodium/high-potassium composition, and it is well-known that a high potassium concentration induces insulin release from islets [39]. It has also been reported that the number of neutrophils in the preserved pancreas is significantly reduced by ETK solution with sivelestat compared with UW solution with sivelestat and that the NE activity is significantly suppressed in ETK solution with sivelestat compared with UW solution with sivelestat [11]. Therefore, we chose to use ETK solution and not UW solution. We also reported that ETK solution with ulinastatin is better for pancreas preservation before islet isolation than HTK solution with ulinastatin due to the differences in terms of energy sources of the solutions [52]. Based on these data, we used ETK solution with ulinastatin for clinical islet transplantation from donation after brain death, donation after circulatory death, and living donation [53].

In this study, 5  $\mu$ M alvelestat was added to the preservation solution based on the data shown in Figure 1. It has been reported that 20  $\mu$ M sivelestat is a suitable concentration for preservation solutions [11]. The IC<sub>50</sub> of sivelestat is 44 nM, and the IC<sub>50</sub> of alvelestat is 12 nM [54], suggesting that the inhibitory effect on NE induced by alvelestat is higher than that induced by sivelestat. Moreover, alvelestat is at least 600-fold more selective than other serin proteases [54].

Inhibition of NE leads to effective treatment, as observed in various preclinical studies including skin, bowel, and lung inflammation, as well as ischemia-reperfusion injury relevant to stroke, myocardial infarction, and transplantation [55]. Clinical trials in cardiovascular and lung disease with NE inhibitors, such as sivelestat and alvelestat, are ongoing. It has been reported that there is a trend towards reduction in sputum inflammatory biomarkers with statistically significant changes in interleukin(IL)-6, regulated

on activation, normal t-cell expressed and secreted (RANTES) and urinary desmosine in alvelestat-treated patients with cystic fibrosis [28] and that significant changes were observed in forced expiratory volume, slow vital capacity, plasma IL-8, and post-waking sputum IL-6 and RANTES levels in alvelestat-treated patients with bronchiectasis [29]. Alvelestat is currently being explored as a potential treatment for  $\alpha$ 1-antitrypsin deficiency-related lung disease [56].

## 5. Conclusions

Supplementation of alvelestat in preservation solutions could improve islet isolation and transplantation. Our results imply that alvelestat has cytoprotective and anti-inflammatory effects through the inhibition of NE activity in the pancreas. Because alvelestat has now been used in a clinical trial, further studies, including the verification of safety and efficacy of alvelestat, are expected. Moreover, experiments with the human pancreas are required before clinical islet transplantation can be applied to treat people with type 1 diabetes.

**Author Contributions:** H.N. designed the experiments and analysed the data; R.O. performed most of the experimental work with the help of C.M.-S., K.K., K.N., Y.T., T.Y., M.S.-Y., I.Y. and M.S.; I.S. and M.W. provided the materials and discussion; M.S. and H.N. contributed to data analysis and interpretation; R.O. and H.N. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The animal study protocol was approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus (protocol code: A2019151, date of approval: 19 March 2020).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author, H.N., upon reasonable request.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Shapiro, A.M.; Lakey, J.R.; Ryan, E.A.; Korbitt, G.S.; Toth, E.; Warnock, G.L.; Kneteman, N.M.; Rajotte, R.V. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **2000**, *343*, 230–238. [[CrossRef](#)] [[PubMed](#)]
2. Shapiro, A.M.; Ricordi, C.; Hering, B.J.; Auchincloss, H.; Lindblad, R.; Robertson, R.P.; Secchi, A.; Brendel, M.D.; Berney, T.; Brennan, D.C.; et al. International trial of the Edmonton protocol for islet transplantation. *N. Engl. J. Med.* **2006**, *355*, 1318–1330. [[CrossRef](#)] [[PubMed](#)]
3. Hering, B.J.; Kandaswamy, R.; Harmon, J.V.; Ansite, J.D.; Clemmings, S.M.; Sakai, T.; Paraskevas, S.; Eckman, P.M.; Sageshima, J.; Nakano, M.; et al. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am. J. Transplant.* **2004**, *4*, 390–401. [[CrossRef](#)] [[PubMed](#)]
4. Froud, T.; Ricordi, C.; Baidal, D.A.; Hafiz, M.M.; Ponte, G.; Cure, P.; Pileggi, A.; Poggioli, R.; Ichii, H.; Khan, A.; et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am. J. Transplant.* **2005**, *5*, 2037–2046. [[CrossRef](#)] [[PubMed](#)]
5. Noguchi, H.; Iwanaga, Y.; Okitsu, T.; Nagata, H.; Yonekawa, Y.; Matsumoto, S. Evaluation of islet transplantation from non-heart beating donors. *Am. J. Transplant.* **2006**, *6*, 2476–2482. [[CrossRef](#)]
6. Abdelli, S.; Ansite, J.; Roduit, R.; Borsello, T.; Matsumoto, I.; Sawada, T.; Allaman-Pillet, N.; Henry, H.; Beckmann, J.S.; Hering, B.J.; et al. Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. *Diabetes* **2004**, *53*, 2815–2823. [[CrossRef](#)]
7. Paraskevas, S.; Maysinger, D.; Wang, R.; Duguid, T.P.; Rosenberg, L. Cell loss in isolated human islets occurs by apoptosis. *Pancreas* **2000**, *20*, 270–276. [[CrossRef](#)]

8. Rosenberg, L.; Wang, R.; Paraskevas, S.; Maysinger, D. Structural and functional changes resulting from islet isolation lead to islet cell death. *Surgery* **1999**, *126*, 393–398. [[CrossRef](#)]
9. Bottino, R.; Balamurugan, A.N.; Tse, H.; Thirunavukkarasu, C.; Ge, X.; Profozich, J.; Milton, M.; Ziegenfuss, A.; Trucco, M.; Piganelli, J.D. Response of human islets to isolation stress and the effect of antioxidant treatment. *Diabetes* **2004**, *53*, 2559–2568. [[CrossRef](#)] [[PubMed](#)]
10. Morini, S.; Braun, M.; Onori, P.; Cicalese, L.; Elias, G.; Gaudio, E.; Rastellini, C. Morphological changes of isolated rat pancreatic islets: A structural, ultrastructural and morphometric study. *J. Anat.* **2006**, *209*, 381–392. [[CrossRef](#)]
11. Machida, T.; Tanemura, M.; Ohmura, Y.; Tanida, T.; Wada, H.; Kobayashi, S.; Marubashi, S.; Eguchi, H.; Ito, T.; Nagano, H.; et al. Significant improvement in islet yield and survival with modified ET-Kyoto solution: ET-Kyoto/Neutrophil elastase inhibitor. *Cell Transplant.* **2013**, *22*, 159–173. [[CrossRef](#)] [[PubMed](#)]
12. Shinichiro, O.; Kuroki, T.; Adachi, T.; Kosaka, T.; Okamoto, T.; Tanaka, T.; Tajima, Y.; Kanematsu, T.; Eguchi, S. The effect of selective neutrophil elastase inhibitor on pancreatic islet yields and functions in rat with hypercytokinemia. *Ann. Transplant.* **2011**, *16*, 99–106. [[CrossRef](#)] [[PubMed](#)]
13. Lewis, E.C.; Shapiro, L.; Bowers, O.J.; Dinarello, C.A. Alpha1-antitrypsin monotherapy prolongs islet allograft survival in mice. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 12153–12158. [[CrossRef](#)]
14. Lewis, E.C.; Mizrahi, M.; Toledano, M.; Defelice, N.; Wright, J.L.; Churg, A.; Shapiro, L.; Dinarello, C.A. alpha1-Antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16236–16241. [[CrossRef](#)]
15. Kono, T.; Okada, S.; Saito, M. Neutrophil elastase inhibitor, sivelestat sodium hydrate prevents ischemia-reperfusion injury in the rat bladder. *Mol. Cell Biochem.* **2008**, *311*, 87–92. [[CrossRef](#)] [[PubMed](#)]
16. Shimoda, M.; Iwasaki, Y.; Okada, T.; Sawada, T.; Kubota, K. Protective effect of Sivelestat in a porcine hepatectomy model prepared using an intermittent Pringle method. *Eur. J. Pharmacol.* **2008**, *587*, 248–252. [[CrossRef](#)]
17. Uchida, Y.; Freitas, M.C.; Zhao, D.; Busuttill, R.W.; Kupiec-Weglinski, J.W. The protective function of neutrophil elastase inhibitor in liver ischemia/reperfusion injury. *Transplantation* **2010**, *89*, 1050–1056. [[CrossRef](#)]
18. Kawabata, K.; Suzuki, M.; Sugitani, M.; Imaki, K.; Toda, M.; Miyamoto, T. ONO-5046, a novel inhibitor of human neutrophil elastase. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 814–820. [[CrossRef](#)]
19. Uchida, Y.; Freitas, M.C.; Zhao, D.; Busuttill, R.W.; Kupiec-Weglinski, J.W. The inhibition of neutrophil elastase ameliorates mouse liver damage due to ischemia and reperfusion. *Liver Transpl.* **2009**, *15*, 939–947. [[CrossRef](#)]
20. Dokai, M.; Madoiwa, S.; Yasumoto, A.; Kashiwakura, Y.; Ishiwata, A.; Sakata, A.; Makino, N.; Ohmori, T.; Mimuro, J.; Sakata, Y. Local regulation of neutrophil elastase activity by endogenous  $\alpha$ 1-antitrypsin in lipopolysaccharide-primed hematological cells. *Thromb. Res.* **2011**, *128*, 283–292. [[CrossRef](#)]
21. Salahuddin, P. Genetic variants of alpha1-antitrypsin. *Curr. Protein Pept. Sci.* **2010**, *11*, 101–117. [[CrossRef](#)] [[PubMed](#)]
22. Stoller, J.K.; Aboussouan, L.S. A review of  $\alpha$ 1-antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.* **2012**, *185*, 246–259. [[CrossRef](#)] [[PubMed](#)]
23. Viglio, S.; Iadarola, P.; D’Amato, M.; Stolk, J. Methods of Purification and Application Procedures of Alpha1 Antitrypsin: A Long-Lasting History. *Molecules* **2020**, *25*, 4014. [[CrossRef](#)] [[PubMed](#)]
24. Ochayon, D.E.; Mizrahi, M.; Shahaf, G.; Baranovski, B.M.; Lewis, E.C. Human  $\alpha$ 1-Antitrypsin Binds to Heat-Shock Protein gp96 and Protects from Endogenous gp96-Mediated Injury In vivo. *Front. Immunol.* **2013**, *4*, 320. [[CrossRef](#)]
25. Stevens, T.; Ekholm, K.; Grånse, M.; Lindahl, M.; Kozma, V.; Jungar, C.; Ottosson, T.; Falk-Håkansson, H.; Churg, A.; Wright, J.L.; et al. AZD9668: Pharmacological characterization of a novel oral inhibitor of neutrophil elastase. *J. Pharmacol. Exp. Ther.* **2011**, *339*, 313–320. [[CrossRef](#)]
26. Delbosc, S.; Rouer, M.; Alsac, J.M.; Louedec, L.; Philippe, M.; Meilhac, O.; Whatling, C.; Michel, J.B. Elastase inhibitor AZD9668 treatment prevented progression of experimental abdominal aortic aneurysms. *J. Vasc. Surg.* **2016**, *63*, 486–492.e1. [[CrossRef](#)]
27. Li, H.; Zhou, X.; Tan, H.; Hu, Y.; Zhang, L.; Liu, S.; Dai, M.; Li, Y.; Li, Q.; Mao, Z.; et al. Neutrophil extracellular traps contribute to the pathogenesis of acid-aspiration-induced ALI/ARDS. *Oncotarget* **2018**, *9*, 1772–1784. [[CrossRef](#)]
28. Elborn, J.S.; Perrett, J.; Forsman-Semb, K.; Marks-Konczalik, J.; Gunawardena, K.; Entwistle, N. Efficacy, safety and effect on biomarkers of AZD9668 in cystic fibrosis. *Eur. Respir. J.* **2012**, *40*, 969–976. [[CrossRef](#)]
29. Stockley, R.; De Soyza, A.; Gunawardena, K.; Perrett, J.; Forsman-Semb, K.; Entwistle, N.; Snell, N. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. *Respir. Med.* **2013**, *107*, 524–533. [[CrossRef](#)]
30. Ricordi, C.; Gray, D.W.; Hering, B.J.; Kaufman, D.B.; Warnock, G.L.; Kneteman, N.M.; Lake, S.P.; London, N.J.; Soggi, C.; Alejandro, R. Islet isolation assessment in man and large animals. *Acta Diabetol. Lat.* **1990**, *27*, 185–195. [[CrossRef](#)]
31. Bank, H.L. Rapid assessment of islet viability with acridine orange and propidium iodide. *In Vitro Cell Dev. Biol.* **1988**, *24*, 266–273. [[CrossRef](#)] [[PubMed](#)]
32. Faust, A.; Rothe, H.; Schade, U.; Lampeter, E.; Kolb, H. Primary nonfunction of islet grafts in autoimmune diabetic nonobese diabetic mice is prevented by treatment with interleukin-4 and interleukin-10. *Transplantation* **1996**, *62*, 648–652. [[CrossRef](#)] [[PubMed](#)]
33. Noguchi, H.; Miyagi-Shiohira, C.; Nakashima, Y.; Saitoh, I.; Watanabe, M. Novel cell-permeable p38-MAPK inhibitor efficiently prevents porcine islet apoptosis and improves islet graft function. *Am. J. Transplant.* **2020**, *20*, 1296–1308. [[CrossRef](#)] [[PubMed](#)]

34. Noguchi, H.; Ueda, M.; Hayashi, S.; Kobayashi, N.; Okitsu, T.; Iwanaga, Y.; Nagata, H.; Nakai, Y.; Matsumoto, S. Ductal injection of preservation solution increases islet yields in islet isolation and improves islet graft function. *Cell Transplant.* **2008**, *17*, 69–81. [[CrossRef](#)]
35. Kuwae, K.; Miyagi-Shiohira, C.; Hamada, E.; Tamaki, Y.; Nishime, K.; Sakai, M.; Yonaha, T.; Makishi, E.; Saitoh, I.; Watanabe, M.; et al. Excellent Islet Yields after 18-h Porcine Pancreas Preservation by Ductal Injection, Pancreas Preservation with MK Solution, Bottle Purification, and Islet Purification Using Iodixanol with UW Solution and Iodixanol with MK Solution. *J. Clin. Med.* **2019**, *8*, 1561. [[CrossRef](#)]
36. Ricordi, C.; Lacy, P.E.; Finke, E.H.; Olack, B.J.; Scharp, D.W. Automated method for isolation of human pancreatic islets. *Diabetes* **1988**, *37*, 413–420. [[CrossRef](#)]
37. Hering, B.J.; Clarke, W.R.; Bridges, N.D.; Eggerman, T.L.; Alejandro, R.; Bellin, M.D.; Chaloner, K.; Czarniecki, C.W.; Goldstein, J.S.; Hunsicker, L.G.; et al. Clinical Islet Transplantation Consortium. Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia. *Diabetes Care* **2016**, *39*, 1230–1240. [[CrossRef](#)]
38. Foster, E.D.; Bridges, N.D.; Feurer, I.D.; Eggerman, T.L.; Hunsicker, L.G.; Alejandro, R.; Clinical Islet Transplantation Consortium. Improved Health-Related Quality of Life in a Phase 3 Islet Transplantation Trial in Type 1 Diabetes Complicated by Severe Hypoglycemia. *Diabetes Care* **2018**, *41*, 1001–1008. [[CrossRef](#)]
39. Noguchi, H.; Ueda, M.; Nakai, Y.; Iwanaga, Y.; Okitsu, T.; Nagata, H.; Yonekawa, Y.; Kobayashi, N.; Nakamura, T.; Wada, H.; et al. Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. *Am. J. Transplant.* **2006**, *6*, 496–504. [[CrossRef](#)]
40. Noguchi, H. Pancreatic Islet Purification from Large Mammals and Humans Using a COBE 2991 Cell Processor versus Large Plastic Bottles. *J. Clin. Med.* **2020**, *10*, 10. [[CrossRef](#)]
41. Nishime, K.; Miyagi-Shiohira, C.; Kuwae, K.; Tamaki, Y.; Yonaha, T.; Sakai-Yonaha, M.; Saitoh, I.; Watanabe, M.; Noguchi, H. Preservation of pancreas in the University of Wisconsin solution supplemented with AP39 reduces reactive oxygen species production and improves islet graft function. *Am. J. Transplant.* **2021**, *21*, 2698–2708. [[CrossRef](#)] [[PubMed](#)]
42. Canelo, R.; Hakim, N.S.; Ringe, B. Experience with histidine tryptophan ketoglutarate versus University Wisconsin preservation solutions in transplantation. *Int. Surg.* **2003**, *88*, 145–151. [[PubMed](#)]
43. Brandhorst, H.; Theisinger, B.; Guenther, B.; Johnson, P.R.; Brandhorst, D. Pancreatic L-Glutamine Administration Protects Pig Islets from Cold Ischemic Injury and Increases Resistance Toward Inflammatory Mediators. *Cell Transplant.* **2016**, *25*, 531–538. [[CrossRef](#)]
44. Ito, T.; Omori, K.; Rawson, J.; Todorov, I.; Asari, S.; Kuroda, A.; Shintaku, J.; Itakura, S.; Ferreri, K.; Kandeel, F.; et al. Improvement of canine islet yield by donor pancreas infusion with a p38MAPK inhibitor. *Transplantation* **2008**, *86*, 321–329. [[CrossRef](#)]
45. Noguchi, H. Regulation of c-Jun NH(2)-Terminal Kinase for Islet Transplantation. *J. Clin. Med.* **2019**, *8*, 1763. [[CrossRef](#)]
46. Land, W.G. The role of posts ischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. *Transplantation* **2005**, *79*, 505–514. [[CrossRef](#)]
47. Pratschke, J.; Wilhelm, M.J.; Kusaka, M.; Basker, M.; Cooper, D.K.; Hancock, W.W.; Tilney, N.L. Brain death and its influence on donor organ quality and outcome after transplantation. *Transplantation* **1999**, *67*, 343–348. [[CrossRef](#)] [[PubMed](#)]
48. Lakey, J.R.; Rajotte, R.V.; Warnock, G.L.; Kneteman, N.M. Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. *Transplantation* **1995**, *59*, 689–694. [[CrossRef](#)]
49. Ricordi, C. Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. *Pancreas* **1991**, *6*, 242–244. [[CrossRef](#)]
50. Barshes, N.R.; Wyllie, S.; Goss, J.A. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: Implications for intrahepatic grafts. *J. Leukoc. Biol.* **2005**, *77*, 587–597. [[CrossRef](#)]
51. Hamada, E.; Ebi, N.; Miyagi-Shiohira, C.; Tamaki, Y.; Nakashima, Y.; Kobayashi, N.; Saitoh, I.; Watanabe, M.; Kinjo, T.; Noguchi, H. Comparison Between Modified Extracellular-Type Trehalose-Containing Kyoto Solution and University of Wisconsin Solution in 18-Hour Pancreas Preservation for Islet Transplantation. *Pancreas* **2018**, *47*, e46–e47. [[CrossRef](#)] [[PubMed](#)]
52. Noguchi, H.; Ueda, M.; Hayashi, S.; Kobayashi, N.; Nagata, H.; Iwanaga, Y.; Okitsu, T.; Matsumoto, S. Comparison of M-Kyoto solution and histidine-tryptophan-ketoglutarate solution with a trypsin inhibitor for pancreas preservation in islet transplantation. *Transplantation* **2007**, *84*, 655–658. [[CrossRef](#)] [[PubMed](#)]
53. Noguchi, H. Clinical Islet Transplantation Covered by Health Insurance in Japan. *J. Clin. Med.* **2022**, *11*, 3977. [[CrossRef](#)]
54. Alvelestat (AZD9668). Available online: <https://www.selleck.co.jp/products/avelestat-azd9668.html> (accessed on 13 July 2022).
55. Henriksen, P.A. The potential of neutrophil elastase inhibitors as anti-inflammatory therapies. *Curr. Opin. Hematol.* **2014**, *21*, 23–28. [[CrossRef](#)]
56. Alvelestat (MPH966) for the Treatment of ALpha-1 ANTitypsin Deficiency (ATALANTa). Available online: <https://clinicaltrials.gov/ct2/show/NCT03679598> (accessed on 13 July 2022).