Copyright © 2012 Cognizant Communication Corporation

digestion. Many neutrophils were activated (red-brown staining) by collagenase digestion in islet isolation using both UW and ET-Kyoto solutions (Fig. 2A), but their number (cells/field) was significantly reduced by the addition of 20 μM of sivelestat in ET-Kyoto solution (UW: 10.2±1.1, S-UW: 6.8±0.4, ET-Kyoto: 9.2±1.3, S-Kyoto: 3.6±0.5) (UW, ET-Kyoto vs. S-Kyoto: $p<0.001$, S-UW vs. S-Kyoto: $p<0.01$) (Fig. 2B). These results suggest a marked increase in enzyme activity by collagenase digestion in all types of isolation solutions (Fig. 2C). However, the enzyme activity was significantly suppressed in S-Kyoto islet isolation than those in other isolations (UW, S-UW and ET-Kyoto-isolation).

Sivelestat suppresses apoptosis in islets after warm digestion during islet isolation

CELL TRANSPLANTATION The Regenerative Medicine Journal

Many TUNEL-positive cells were identified in the islets at the end of warm digestion (Fig. 3A), whereas no such cells were detected before digestion (data not shown). Quantitative analysis indicated a significantly lower number of TUNEL-positive cells within the islets of the S-Kyoto group (6.6±1.5) compared with those of UW, S-UW, and ET-Kyoto groups (23.1±3.9, 20.8±5.0, and 15.3±2.6, respectively) ($p<0.001$, each) (Fig. 3B).

Improvement of islet yield elicited by islet isolation with S-Kyoto solution

To investigate the beneficial effects of sivelestat in islet isolation, islet isolations were performed using either UW or ET-Kyoto solution, with or without 20 μM of sivelestat. Islet yield, isolation index and islet purity after purification were significantly improved by S-Kyoto islet isolation (Table 1). The recovery rate of

Copyright © 2012 Cognizant Communication Corporation

purification was also significantly improved by S-Kyoto islet isolation, compared to those by other isolation solutions (UW: 55.8±10.4%, S-UW: 56.6±12.7%, ET-Kyoto: 56.6±4.6%, S-Kyoto: 76.0±5.2%). Based on the results of UW isolation groups (UW, S-UW), no beneficial effects of sivelestat were elicited under these islet isolation parameters. With regard to islet size distribution, the percentage of large islets (>150 nm in diameter) in the S-Kyoto group was markedly higher than that in other isolation groups (Fig. 4A).

To further examine the morphological changes in isolated islets, SEM was employed. As shown in Figure 4B, islets obtained from the UW groups (UW, S-UW) were poor, based on the irregular islet surface and detection of islet damage during the isolation procedure. Similarly, islets obtained from ET-Kyoto isolation had irregular shape. On the other hand, islets isolated by S-Kyoto solution were well preserved, with round and smooth surface.

CELL TRANSPLANTATION The Regenerative Medicine Journal

Improvement of islet viability elicited by islet isolation with S-Kyoto solution

Next, we evaluated islet viability using fluorescence labeling with TMRE and 7-AAD (Fig. 5). In the UW groups (UW, S-UW), no significant difference was noted in islet viability assay using TMRE and 7-AAD between with or without sivelestat (UW (n=5) vs. S-UW (n=5) (60.0±5.6% vs. 63.3±4.4%, not significant). However, the percentage of dead cells, represented by 7-AAD-positive cells, was significantly reduced in S-UW isolation group [UW (n=5) vs. S-UW (n=5), 25.8±5.3% vs. 17.2±1.3%, $p<0.05$]. In contrast, the viability of islets isolated with S-Kyoto solution was significantly better relative to that of ET-Kyoto isolation [ET-Kyoto (n=5) vs. S-

Copyright © 2012 Cognizant Communication Corporation

Kyoto (n=5), 67.0±1.2% vs. 75.4±2.0%, $p<0.001$]. Moreover, the percentage of dead cells in the S-Kyoto group was also lower compared with that in ET-Kyoto isolation [ET-Kyoto (n-5) vs. S-Kyoto (n=5), 18.6±2.2% vs. 11.6±2.4%, $p<0.01$]. Similar findings of islet viability were noted by MTS assay (Fig. 6A). The highest viability of fresh islets was noted with S-Kyoto solution [ET-Kyoto (n=5) vs. S-Kyoto (n=5), 0.172±0.013 vs. 0.206±0.026, $p<0.05$, Fig. 6A]. With regard to the *in vitro* culture after islet isolation, although islet viability in each group decreased gradually, that of the S-Kyoto group was well preserved compared with other isolation groups (Fig. 6B).

Improvement of islet function elicited by islet isolation with S-Kyoto solution

Next, we evaluated insulin function of islets using static glucose change. Although no significant difference was observed in insulin concentration under low glucose (2.8 mM) using each solution, insulin concentration under high glucose (20 mM) and the SI improved significantly by S-Kyoto islet isolation in comparison with those of other isolation solutions (Table 2). Based on the results of UW isolation groups (UW, S-UW), no beneficial effects of sivelestat were elicited as determined by the SI.

CELL TRANSPLANTATION The Regenerative Medicine Journal

Prolongation of islet graft survival in S-Kyoto group and in in vivo experiments

Transplantation of 500 allogeneic islets of the ET-Kyoto group was associated with graft survival of 7.4±1.1 days (Fig. 7A, Table 3). A significant prolongation of islet graft survival was noted after transplantation of the S-Kyoto group, compared with islet grafts of the ET-Kyoto group (11.2±1.3 days, $p<0.05$). Importantly, islet

Copyright © 2012 Cognizant Communication Corporation

transplantation coupled with 15-day intraperitoneal administration of 2 mg/day sivelestat elicited significant prolongation of graft survival, compared with both ET-Kyoto and S-Kyoto groups, respectively (Figure 7A, Table 3). Next, NE activity in sera of recipient mice was measured. NE activity increased gradually after islet transplantation in the S-Kyoto group. However, sivelestat significantly suppressed the enzyme activity measured at day 28 after transplantation (Fig. 7B). These results suggest that prolongation of islet graft survival in the sivelestat ip group is due to inhibition of NE enzyme activity and the anti-inflammatory properties of sivelestat.

To examine islet graft function *in vivo*, IPGTT was performed at day 7 after transplantation. In non-diabetic wild-type mice, injection of glucose induced hyperglycemia with the peak blood glucose level recorded at 15 min, but the level returned to normal at 60-120 min after the injection (Fig. 7C). A similar pattern was observed in mice transplanted with S-Kyoto and S-Kyoto and treated with sivelestat. On the other hand, blood glucose levels of mice transplanted with ET-Kyoto group and untreated diabetic mice were significantly higher than those of the control wild-type mice before injection and at 15, 60 and 120 min after injection of glucose (Fig. 7C). Immunohistochemical staining for insulin confirmed that islet transplantation with S-Kyoto and S-Kyoto with sivelestat prolonged graft survival. Small islet grafts with many infiltrating inflammatory cells were detected at day 7 after transplantation in the ET-Kyoto group, whereas insulin-positive islet grafts with well preserved islet structure were found in the S-Kyoto and S-Kyoto with sivelestat groups (Fig. 7D).

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

Sivelestat suppresses proinflammatory cytokines after warm digestion during islet isolation

We measured the levels of proinflammatory cytokines (IL-2, 4, 6, 10, 17A, IFN-γ, TNF-α) in the isolation solution at each step of islet isolation, including before warm digestion, at the end of warm digestion and after purification. The levels at the end of warm digestion were markedly higher than before warm digestion (Fig. 8). On the other hand, IL-6 and TNF-α level were significantly lower at the end of warm digestion in S-Kyoto solution compared with other isolation solutions such as UW and ET-Kyoto (Fig. 8).

Sivelestat suppresses proinflammatory cytokines in serum of islet recipients after transplantation

CELL TRANSPLANTATION The Regenerative Medicine Journal

The serum levels of IL-6 and TNF-α in islet recipients were significantly higher at 7 and 14 post-transplantation days than before transplantation (day -1), whereas no significant increases were noted in the levels of other cytokines except IL-6 and TNF-α (Fig. 9). The serum levels of IL-6 and TNF-α were significantly lower in the sivelestat intraperitoneal (ip) group compared with non-sivelestat ip group at 7 and 14 post-transplantation days (Fig. 9).

Copyright © 2012 Cognizant Communication Corporation

DISCUSSION

Islet transplantation is currently one of the most attractive strategies for the treatment of type 1 diabetes. One important key to achieving successful insulin-independence after islet transplantation is acquiring a sufficient donor islet mass (1,10). The number and quality of islets recovered from isolation are influenced by several factors (10). Tissue damage of donor pancreas starts to occur as early as the onset of brain death associated with hypotension, peripheral vasoconstriction, tissue ischemia, and release of stress hormones and inflammatory mediators (10,20,24,38). Moreover, at the time of procurement, donor pancreas is exposed to warm ischemia after donor cross-clamping and cold ischemia storage in preservation solution, such as UW, ET-Kyoto solution (23,40). Furthermore, during islet isolation, warm digestion, trauma and hypoxia, may cause cell damage in isolated islets similar to ischemia/reperfusion injury (IRI) of other transplant organs (10). Therefore, we need to design an efficient isolation method to reduce cell damage toward donor pancreata.

The major finding of the present study was identification of the crucial role of NE in islet isolation and transplantation. First, we showed a marked increase in NE activity during islet isolation, especially at the end of warm digestion by collagenase (Fig. 2C), and that NE was cytotoxic against isolated islets (Fig. 1A). Second, the addition of sivelestat to the isolation solution during islet isolation inhibited NE activity (Fig. 2C) and significantly improved islet yields, islet viability and insulin function of isolated islets (Tables 1 and 2). Furthermore, we also showed that

Copyright © 2012 Cognizant Communication Corporation

treatment of recipient animals with sivelestat significantly prolonged the survival of insulin secreting islet allografts (Fig. 7A, Table 3)

In clinical liver transplantation, IRI, an exogenous antigen-independent inflammatory event, remains a major problem, because IRI causes early transplant graft failure and can lead to a higher incidence of both acute and chronic rejections (6,54,55). The mechanisms underlying liver IRI involve leukocyte accumulation and activation of neutrophils, Kupffer cells (macrophages), and T cells, secretion of proinflammatory cytokines and chemokines, complement activation and activation of vascular cell adhesion molecules (49,51,53-55). Recent reports also indicated that the cross-talk interaction between NE and toll like receptor (TLR) 4 promotes liver damage, and that NE inhibitors, such as sivelestat, ameliorated the hepatocellular damage by reducing the expression of proinflammatory cytokines, chemokines, and TLR4 (24,54,55).

CELL TRANSPLANTATION The Regenerative Medicine Journal

In islet transplantation, proinflammatory cytokines such as TNF-α and IL-1β induce β-cell apoptosis (1,3,27). These proinflammatory cytokines are produced by acinar and ductal cells (10). Moreover, in isolated islets, resident macrophages and monocytes produce cytokines (3,10,25). During islet isolation, the pancreatic tissues are warmed up to 37°C for collagenase digestion (10). As shown in Figure 2, many neutrophils were activated and released NE at the end of warm digestion. The released NE caused injury to membrane components of macrophages, acinar cells and islets. Activated macrophages and acinar cells produce proinflammatory cytokines, including TNF-α and IL-6 (3). In fact, significantly high levels of TNF-α and IL-6 were found in isolation solution at the end of warm digestion compared with those before warm

Copyright © 2012 Cognizant Communication Corporation

digestion (Fig. 8). Moreover, NE may also serve as a putative endogenous TLR4 ligand, causing TLR4 up-regulation on macrophages and islets during the isolation process (5,39,54,55). Such excessive expression of TNF-α and TLR 4 affects the surrounding islets causing their apoptosis (7,19). Indeed, to detect apoptotic cells during islet isolation, TUNEL staining was performed before and at the end of warm digestion. Many TUNEL-positive cells were detected in UW solution at the end of warm digestion, whereas no such cells were detected in pancreatic tissue before warm digestion (Fig. 3A). What is the mechanism of NE-induced islet injury ? Sivelestat effectively prevented the cross-talk between NE and inflammation responses, including the expression of proinflammatory cytokines (TNF-α, IL-6) and TLR4, resulting in a significant improvement in islet yields, islet viability and insulin function in islet isolation with S-Kyoto solution. Furthermore, analysis of serum cytokine production profile (IL-2, 4, 6, 10, 17A, IFN-γ, TNF-α) in islet recipient mice showed significant increase in IL-6 and TNF-α production in serum at 1 and 2 weeks after islet transplantation, compared with those before islet transplantation (day -1), whereas no such increase was observed in the production of other cytokines before/after islet transplantation (Fig. 9). As shown in Figure 7B, NE activity increased gradually after islet transplantation. However, sivelestat significantly suppressed NE activity and the increase in IL-6 and TNF-α production in mice (Fig. 7B and 9). Taken together, local inflammatory reaction, resulting in functional loss of islet grafts occurred after transplantation and treatment with sivelestat inhibited such inflammatory reactions, as evidenced by reduced IL-6 and TNF-α production, and resulted in prolongation of islet allograft survival.

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

Trypsin released from pancreatic acinar cells directly destroys islets (35). Previous studies demonstrated that inhibition of trypsin by pefabloc or ulinastatin during pancreas digestion improved islet yield and reduced the fraction of embedded islets (22,35). These findings suggest that trypsin may degrade pancreatic ductules, resulting in reduced delivery of collagenase solution (35). However, the molecular weight of the new recombinant NE inhibitor sivelestat is much lower than alpha-1 protease inhibitors, and appears to exert its cytoprotective effect in the microenvironment between neutrophil and pancreatic tissues (12,30). In this study, we examined the effects of the addition of sivelestat to ET-Kyoto solution. ET-Kyoto solution has a high sodium/low potassium composition with comparatively low viscosity (35). Therefore, it allows sufficient organ flushing after harvesting the pancreatic tissues (28,35). Furthermore, we also examined the effects of the addition of sivelestat to the UW solution. The results suggested that sivelestat also provided cytoprotection when added to the UW solution. This conclusion was based on the finding that the decrease in the number of activated neutrophils and neutrophil elastase activity in the S-UW group tended to be larger than in the UW group (Fig. 2B and 2C), albeit statistically insignificant. The islets isolated from the UW solution are easily damaged by mechanical stress due to the high viscosity of the UW solution (13). Indeed, in our experiment, membrane shear damage had a negative impact on the isolated islets of the UW group during the isolation process, as shown in Figure 4B. This was probably due to one or more of the following reasons: 1) higher viscosity of the UW solution relative to that of the ET-Kyoto solution, 2) the high percentage of activated neutrophils present in the pancreatic tissue, and 3) the higher

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

level of neutrophil elastase released in the UW than in ET-Kyoto. We speculate that the balance between the amount of released neutrophil elastase and its inhibition by collagenase digestion tilts towards excess elastase activity when sivelestat is added to the UW solution. We also assessed the synergistic effects of ulinastatin and sivelestatin on improvement of islet yield and islet viability. However, no additive effects were observed in comparison with S-Kyoto solution alone and S-Kyoto solution with ulinastatin (data not shown).

As shown in Figure 4B, SEM showed well preserved islets that were isolated by S-Kyoto solution, as evident by their round and smooth surface. NE is reported to increase the permeability of vascular endothelial cells, a process known to be involved in tissue edema (30). Indeed, during islet isolation, inhibition of NE activity by sivelestat may maintain cell membrane stability and permeability of endothelial cells in isolated islets, preventing tissue edema, and leading to improvement of islet yield and insulin function of isolated islets.

CELL TRANSPLANTATION The Regenerative Medicine Journal

In conclusion, we succeeded in isolating large numbers of islets using a new preservation solution, S-Kyoto solution, and in significant prolongation of islet graft survival in recipient mice treated with sivelestat. Our results emphasize the role of NE in the pathophysiology of islet damage during islet isolation and after transplantation. NE contributes to the accumulation of neutrophils and secretion of proinflammatory mediators during the isolation procedure and after islet transplantation. Therefore, treatment with NE inhibitors is potentially suitable for better harvest of transplantable islets and long-term islet allograft survival, allowing successful management of diabetes with islets from a single donor. We plan to assess in the near future the

Copyright © 2012 Cognizant Communication Corporation

beneficial effects of S-Kyoto solution and monotherapy with sivelestat in human islet transplantation.

ACKNOWLEDGMENTS: The authors thank Dr. F.G. Issa (www.word-medex.com.au) for the careful reading and editing of this manuscript, ONO pharmaceutical Co. for kindly providing sivelestat and Otsuka pharmaceutical Co. for generously providing ET-Kyoto solution.

DISCLOSURES: The authors declare no conflict of interest.

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

REFERENCES

1. Abdelli, S.; Ansite, J.; Roduit, R.; Borsello, T.; Matsumoto, I.; Sawada, T.; Allaman-Pillet, N.; Henry, H.; Beckmann, J.S.; Hering B.J.; Bonny, C. Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. Diabetes 53: 2815-2823; 2004.
2. Andrikopoulos, S.; Blair, A.R.; Deluca, N.; Fam, B.C.; Proietto, J. Evaluating the glucose tolerance test in mice. Am. J. Physiol. Endocrinol. Metab. 295: E1323-1332; 2008.
3. Barshes, N.R.; Wyllie, S.; Goss, J.A. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. J. Leukoc. Biol. 77: 587-597; 2005.
4. 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 53: 2559-2568; 2004.
5. Devaney, J.M.; Greene, C.M.; Taggart, C.C.; Carroll, T.P.; O'Neill, S.J.; McElvaney, N.G.; Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. FEBS Lett. 544: 129-132; 2003.
6. Farmer, D.G.; Amersi, F.; Kupiec-Weglinski, J.; Busuttil, R.W. Current status of ischemia and reperfusion injury in the liver. Transplant. Rev. 14: 106-126; 2000.
7. Gao, Q.; Ma, L.L.; Gao, X.; Yan, W.; Williams, P.; Yin, D.P. TLR 4 mediates early graft failure after intraportal islet transplantation. Am. J. Transplant. 10: 1588-

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

1596; 2010.

8. Hering, B.J. Achieving and maintaining insulin independence in human islet transplant recipients. Transplantation.79: 1296-1297; 2005.
9. Ichii, H.; Pileggi, A.; Molano, R.D.; Baidal, D.A.; Khan, A.; Kuroda, Y.; Inverardi, L.; Goss, J.A.; Alejandro, R.; Ricordi, C. Rescue purification maximizes the use of human islet preparations for transplantation. Am. J. Transplant. 5: 21-30; 2005.
10. Ito, T.; Omori, K.; Rawson, J.; Todorov, I.; Asari, S.; Kuroda, A.; Shintaku, J.; Itakura, S.; Ferreri, K.; Kandeel, F.; Mullen, Y. Improvement of canine islet yield by donor pancreas infusion with a p38 MAPK inhibitor. Transplantation. 86: 321-329; 2008.
11. Kawabata, K.; Hagio, T.; Matsumoto, S.; Nakao, S.; Orita, S.; Aze, Y.; Ohno, H. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. Am. J. Respir. Crit. Care Med. 161: 2013-2018; 2000.
12. 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. 17: 814-820; 1991.
13. Kenmochi, T.; Miyamoto, M.; Sasaki, H.; Une, S.; Nakagawa, Y.; Moldovan, S.; Benhamou, P.Y.; Brunicardi, F.C.; Tanaka, H.; Mullen, Y. LAP-1 cold preservation solution for isolation of high-quality human pancreatic islets. Pancreas 17: 367-377; 1998.
14. Khandoga, A.; Huettinger, S.; Khandoga, A.G.; Li, H.; Butz, S.; Jauch, K.W.; Vestweber, D.; Krombach, F. Leukocyte transmigration in inflamed liver: A role

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

for endothelial cell-selective adhesion molecule. J. Hepatol. 50: 755-765; 2009.

15. Kin, T.; Johnson, P.R.; Shapiro, A.M.; Lakey, J.R. Factors influencing the collagenase digestion phase of human islet isolation. Transplantation. 83:7-12; 2007.
16. Kin, T.; Zhai, X.; Murdoch, T.B.; Salam, A.; Shapiro, A.M.; Lakey, J.R. Enhancing the success of human islet isolation through optimization and characterization of pancreas dissociation enzyme. Am. J. Transplant. 7: 1233-1241; 2007.
17. Kono, T.; Okada, S.; Saito, M. Neutrophil elastase inhibitor, sivelestat sodium hydrate prevents ischemia-reperfusion injury in the rat bladder. Mol. Cell. Biochem. 311: 87-92; 2008.
18. Korsgren, O.; Lundgren, T.; Felldin, M.; Foss, A.; Isaksson, B.; Permert, J.; Persson, N.H.; Rafael, E.; Rydén, M.; Salmela, K.; Tibell, A.; Tufveson, G.; Nilsson, B. Optimising islet engraftment is critical for successful clinical islet transplantation. Diabetologia 51: 227-232; 2008.
19. Krüger, B.; Yin, N.; Zhang, N.; Yadav, A.; Coward, W.; Lal, G.; Zang, W.; Heeger, P.S.; Bromberg, J.S.; Murphy, B.; Schröppel, B. Islet-expressed TLR 2 and TLR 4 sense injury and mediate early graft failure after transplantation. Eur. J. Immunol. 40: 2914-2924; 2010.
20. Kusaka, M.; Pratschke, J.; Wilhelm, M.J.; Ziai, F.; Zandi-Nejad, K.; Mackenzie, H.S.; Hancock, W.W.; Tilney, N.L. Activation of inflammatory mediators in rat renal isografts by donor brain death. Transplantation 69: 405-410; 2000.
21. Kwon, A.H.; Qiu, Z. Neutrophil elastase inhibitor prevents endotoxin-induced liver injury following experimental partial hepatectomy. Br. J. Surg. 94: 609-619;

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

2007.

22. Lakey, J.R.; Helm, L.M.; Kin, T.; Korbutt, G.S.; Rajotte, R.V.; Shapiro, A.M.; Warnock, G.L. Serine-protease inhibition during islet isolation increases islet yield from human pancreases with prolonged ischemia. Transplantation 72: 565-570; 2001.

23. Lakey, J.R.; Rajotte, R.V.; Warnock, G.L.; Kneteman, N.M. Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. Transplantation 59: 689-694; 1995.

24. Land, W.G. The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. Transplantation 79: 505-514; 2005.

25. Lawrence, M.C.; Naziruddin, B.; Levy, M.F.; Jackson, A.; McGlynn, K. Calcineurin/nuclear factor of activated T cells and MAPK signaling induce TNF-{alpha} gene expression in pancreatic islet endocrine cells. J. Biol. Chem. 286: 1025-1036; 2011.

26. Li, D.S.; Yuan, Y.H.; Tu, H.J.; Liang, Q.L.; Dai, L.J. A protocol for islet isolation from mouse pancreas. Nat. Protoc. 4: 1649-1652; 2009.

27. Linn, T.; Schmitz, J.; Hauck-Schmalenberger, I.; Lai, Y.; Bretzel, R.G.; Brandhorst, H.; Brandhorst, D. Ischemia is linked to inflammation and induction of angiogenesis in pancreatic islets. Clin. Exp. Immunol. 144: 179-187; 2006.

28. Matsumoto, S.; Noguchi, H.; Shimoda, M.; Ikemoto, T.; Naziruddin, B.; Jackson, A.; Tamura, Y.; Olson, G.; Fujita, Y.; Chujo, D.; Takita, M.; Kobayashi, N.; Onaca, N.; Levy, M. Seven consecutive successful clinical islet isolations with pancreatic

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

ductal injection. Cell Transplant. 19: 291-297; 2010.

29. Moberg, L.; Korsgren, O.; Nilsson, B. Neutrophil granulocytes are the predominant cell type infiltrating pancreatic islets in contact with ABO-compatible blood. Clin. Exp. Immunol. 142: 125-131; 2005.

30. Mori, H.; Nagahiro, I.; Osaragi, T.; Kotani, K.; Nakanishi, H.; Sano, Y.; Date, H.; Shimizu, N. Addition of a neutrophil elastase inhibitor to the organ flushing solution decreases lung reperfusion injury in rat lung transplantation. Eur. J. Cardiothorac. Surg. 32: 791-795; 2007.

31. 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. 209: 381-392; 2006.

32. Morohoshi, Y.; Matsuoka, K.; Chinen, H.; Kamada, N.; Sato, T.; Hisamatsu, T.; Okamoto, S.; Inoue, N.; Takaishi, H.; Ogata, H.; Iwao, Y.; Hibi, T. Inhibition of neutrophil elastase prevents the development of murine dextran sulfate sodium-induced colitis. J. Gastroenterol. 41: 318-324; 2006.

33. Noguchi, H.; Nakai, Y.; Matsumoto, S.; Kawaguchi, M.; Ueda, M.; Okitsu, T.; Iwanaga, Y.; Yonekawa, Y.; Nagata, H.; Minami, K.; Masui, Y.; Futaki, S.; Tanaka, K. Cell Permeable peptide of JNK inhibitor prevents islet apoptosis immediately after isolation and improves islet graft function. Am. J. Transplant. 5: 1848-1855; 2005.

34. Noguchi, H.; Naziruddin, B.; Onaca, N.; Jackson, A.; Shimoda, M.; Ikemoto, T.; Fujita, Y.; Kobayashi, N.; Levy, M.F.; Matsumoto, S. Comparison of modified Celsior solution and M-Kyoto solution for pancreas preservation in human islet

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

isolation. Cell Transplant. 19: 751-758; 2010.

35. Noguchi, H.; Ueda, M.; Nakai, Y.; Iwanaga, Y.; Okitsu, T.; Nagata, H.; Yonekawa, Y.; Kobayashi, N.; Nakamura, T.; Wada, H.; Matsumoto, S. Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. Am. J. Transplant. 6: 496-504; 2006.

36. Ohmura, Y.; Tanemura, M.; Kawaguchi, N.; Machida, T.; Tanida, T.; Deguchi, T.; Wada, H.; Kobayashi, S.; Marubashi, S.; Eguchi, H.; Takeda, Y.; Matsuura, N.; Ito, T.; Nagano, H.; Doki, Y.; Mori, M. Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice. Transplantation 90: 1366-1373; 2010.

37. Paraskevas, S.; Maysinger, D.; Wang, R.; Duguid, T.P.; Rosenberg, L. Cell loss in isolated human islets occurs by apoptosis. Pancreas 20: 270-276; 2000.

CELL TRANSPLANTATION The Regenerative Medicine Journal

38. 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 67: 343-348; 1999.

39. Ribeiro-Gomes, F.L.; Moniz-de-Souza, M.C.; Alexandre-Moreira, M.S.; Dias, W.B.; Lopes, M.F.; Nunes, M.P.; Lungarella, G.; Dos Reis, G.A. Neutrophils activate macrophages for intracellular killing of *Leishmania major* through recruitment of TLR 4 by neutrophil elastase. J. Immunol. 179: 3988-3994; 2007.

40. Ricordi, C. Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. Pancreas 6: 242-244; 1991.

41. Ricordi, C.; Strom, T.B. Clinical islet transplantation: advances and immunological challenges. Nat. Rev. Immunol. 4: 259-268; 2004.

Copyright © 2012 Cognizant Communication Corporation

42. Rosenberg, L.; Wang, R.; Paraskevas, S.; Maysinger, D. Structural and functional changes resulting from islet isolation lead to islet cell death. Surgery 126: 393-398; 1999.

43. Ryan, E.A.; Lakey, J.R.; Paty, B.W.; Imes, S.; Korbutt, G.S.; Kneteman, N.M.; Bigam, D.; Rajotte, R.V.; Shapiro, A.M. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes 51: 2148-2157; 2002.

44. Ryan, E.A.; Lakey, J.R.; Rajotte, R.V.; Korbutt, G.S.; Kin, T.; Imes, S.; Rabinovitch, A.; Elliott, J.F.; Bigam, D.; Kneteman, N.M.; Warnock, G.L.; Larsen, I.; Shapiro, A.M. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 50: 710-719; 2001.

45. Ryan, E.A.; Paty, B.W.; Senior, P.A.; Bigam, D.; Alfadhli, E.; Kneteman, N.M.; Lakey, J.R.; Shapiro, A.M. Five-year follow-up after clinical islet transplantation. Diabetes 54: 2060-2069; 2005.

46. Shapiro, A.M.; Lakey, J.R.; Paty, B.W.; Senior, P.A.; Bigam, D.L.; Ryan, E.A. Strategic opportunities in clinical islet transplantation. Transplantation 79: 1304-1307; 2005.

47. Shapiro, A.M.; Lakey, J.R.; Ryan, E.A.; Korbutt, 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. 343: 230-238; 2000.

48. 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.; Cagliero, E.;

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

Alejandro, R.; Ryan, E.A.; DiMercurio, B.; Morel, P.; Polonsky, K.S.; Reems, J.A.; Bretzel, R.G.; Bertuzzi, F.; Froud, T.; Kandaswamy, R.; Sutherland, D.E.; Eisenbarth, G.; Segal, M.; Preiksaitis, J.; Korbutt, G.S.; Barton, F.B.; Viviano, L.; Seyfert-Margolis, V.; Bluestone, J.; Lakey, J.R. International trial of the Edomonton protocol for islet transplantation. N. Engl. J. Med. 355:1318-1330; 2006.

49. 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. 587: 248-252; 2008.

50. Tamakuma, S.; Ogawa, M.; Aikawa, N.; Kubota, T.; Hirasawa, H.; Ishizaka, A.; Tanaka, N.; Hamada, C.; Matsuoka, S.; Abiru, T. Relationship between neutrophil elastase and acute lung injury in humans. Pulm. Pharmacol. Ther. 17: 271-279; 2004.

51. Teoh, N.C.; Farrell, G.C. Hepatic ischemia reperfusion injury: Pathogenic mechanisms and basis for hepatoprotection. J. Gastroenterol. Hepatol. 18: 891-902; 2003.

52. Thomas, F.; Wu, J.; Contreras, J.L.; Smyth, C.; Bilbao, G.; He, J.; Thomas, J. A tripartite anoikis-like mechanism causes early isolated islet apoptosis. Surgery 130: 333-338; 2001.

53. Tsuchihashi, S.; Ke, B.; Kaldas, F.; Flynn, E.; Busuttil, R.W.; Briscoe, D.M.; Kupiec-Weglinski, J.W. Vascular endothelial growth factor antagonist modulates leukocyte trafficking and protects mouse livers against ischemia/reperfusion injury. Am. J. Pathol. 168: 695-705; 2006.

CELL TRANSPLANTATION The Regenerative Medicine Journal

Copyright © 2012 Cognizant Communication Corporation

54. Uchida, Y.; Freitas, M.C.; Zhao, D.; Busuttil, R.W.; Kupiec-Weglinski, J.W. The inhibition of neutrophil elastase ameliorates mouse liver damage due to ischemia and reperfusion. Liver Transpl. 15: 939-947; 2009.

55. Uchida, Y.; Freitas, M.C.; Zhao, D.; Busuttil, R.W.; Kupiec-Weglinski, J.W. The protective function of neutrophil elastase inhibitor in liver Ischemia/reperfusion injury. Transplantation 89: 1050-1056; 2010.

56. Wang, P.; Henning, S.M.; Heber, D. Limitations of MTT and MTS-based assays for measurement of antiproliferative activity of green tea polyphenols. PLoS One 5: e10202; 2010.

57. Wu, Y.; Han, B.; Luo, H.; Roduit, R.; Salcedo, T.W.; Moore, P.A.; Zhang, J.; Wu, J. DcR3/TR6 effectively prevents islet primary nonfunction after transplantation. Diabetes 52: 2279-2286; 2003.

58. Yin, D.; Ding, J.W.; Shen, J.; Ma, L.; Hara, M.; Chong, A.S. Liver ischemia contributes to early islet failure following intraportal transplantation: benefits of liver ischemic-preconditioning. Am. J. Transplant.6: 60-68; 2006.

59. Yoshimura, K.; Nakagawa, S.; Koyama, S.; Kobayashi, T.; Homma, T. Roles of neutrophil elastase and superoxide anion in leukotriene B4-induced lung injury in rabbit. J. Appl. Physiol. 76: 91-96; 1994.

60. Zeiher, B.G.; Artigas, A.; Vincent, J.L.; Dmitrienko, A.; Jackson, K.; Thompson, B.T.; Bernard, G. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. Crit. Care Med. 32: 1695-1702; 2004.

CELL TRANSPLANTATION The Regenerative Medicine Journal