Activation of c-Jun NH$_2$-terminal Kinase during Islet Isolation

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Abstract. Pancreatic islet transplantation has been remarkably improved by the Edmonton protocol; however, it is not easy to achieve insulin independence after islet transplantation from one donor pancreas. The islet isolation procedure itself destroys cellular and noncellular components of the pancreas that probably play a role in supporting islet survival. Further islet transplantation exposes cells to a variety of stressful stimuli such as proinflammatory cytokines. The reduction in islet mass immediately after isolation and transplantation implicates β cell death by apoptosis and the prerecruitment of intracellular death signalling pathways. The c-Jun NH$_2$-terminal kinases (JNKs) are classic stress-activated protein kinases and many cellular stresses have been shown to stimulate JNK activation. JNK in the pancreas is activated during brain death, pancreas procurement, and organ preservation, and its activity is progressively increased during the isolation procedure. Moreover, JNK activity in the transplanted liver after islet transplantation increases markedly within 24 hrs. Use of the JNK inhibitor in pancreas preservation, islet culture, and/or islet transplantation prevents islet apoptosis and improves islet graft function. These findings suggest that the control of JNK activation is important for pancreatic islet transplantation.

Key words: Islet transplantation, c-Jun NH$_2$-terminal kinase, Apoptosis, Proinflammatory cytokine, Protein transduction technology

Type 1 diabetes results from a progressive decrease in β-cell mass and function by autoimmunity [1]. Pancreatic islet transplantation may successfully restore normoglycemia in type 1 diabetic patients. Application of the Edmonton protocol has markedly improved the outcome [2–5] but the insulin independence rate after islet transplantation from one donor pancreas has been found to remain low, suggesting that successful grafting requires the transplantation of a sufficient number of islets, usually from two or more donors. The low frequency of islet grafting is dependent on poor islet recovery from donors [6] and early islet loss during the first hours following grafting, termed primary graft nonfunction [7, 8]. Indeed, the islet isolation procedure itself destroys cellular and noncellular components of the pancreas that probably play a role in supporting islet survival [8, 9]. Furthermore, islet transplantation exposes cells to a variety of stressful stimuli, notably proinflammatory cytokines that encourage β cell death and lead to early graft failure [10–12]. The reduction in islet mass immediately after isolation and transplantation implicates β cell death by apoptosis and the prerecruitment of intracellular death signaling pathways [13–15].

Islet transplantation involves the exposure of islets to several forms of stress, not only as the result of pancreas preservation and islet isolation, but also inflammation and glucose toxicity after transplantation. The group of mitogen-activated protein kinases (MAPKs) [16, 17] is one of the most important factors in death-signaling pathways that have been shown to contribute to β-cell death in vitro. MAPKs are expressed in all eukaryotic cells, are activated by diverse stimuli ranging from cytokines, growth factors, neurotransmitters, hormones, cellular stress, and cell adherence. Three major conserved groups of MAPKs have been described: extracellular signal-regulated kinases (ERKs;
ERK1/2/3) [18], p38 kinases (p38 α/β/γ/δ) [19], and c-Jun NH2-terminal kinases (JNKs; JNK1/2/3) [20]. In mammals, these MAPKs can be activated by six MAPK kinases (MKKs): MKK1 and -2 are upstream activators of ERK1/2 [21], MKK3, -4 and -6 are activators of p38 [22–24], and MKK4 and -7 regulate JNK [25, 26]. Both JNK and p38, called “stress kinases”, are similarly activated by multiple stresses, including ultraviolet and γ-irradiation, cytotoxic drugs, cold and heat shock, loss of survival factors, hypo- and hyperosmolarity, proinflammatory cytokines, shearing stresses, and reactive oxygen species [27, 28]. Both JNK and p38 activate downstream nuclear transcription factors that participate in the cellular response [29, 30], including activation of the activator protein-1 (AP-1), which is formed of heteromers of c-fos, c-Jun, and ATF-2 and is required for some forms of apoptosis, notably in neuronal cells [31, 32]. In type 1 diabetes, JNK plays a central role in intracellular events that signal β-cell loss after exposure to proinflammatory cytokine interleukin (IL)-1β [16, 33]. JNK is abnormally elevated in various tissues under diabetic conditions due to the phenomenon known as “glucose toxicity” and it has been demonstrated that activation of the JNK pathway interferes with insulin action [34, 35] and β-cell function [36, 37] as well as insulin bio-synthesis [36]. A major transcriptional target of JNK and p38 is the c-fos gene.

Brain death and pancreas preservation

It is recognized that brain death defines the effects on hemodynamic stability, hormone regulation, and inflammatory reactivity. It has been demonstrated that, following brain death, organs deteriorate not only by the effect of massive acute cerebral injury but also hypotension and circulating factors [38, 39]. Brain death is characterized by extensive cortical necrosis that stimulate multiple cell types to release proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon-γ (IFN-γ) [38, 40–43]. In the pancreas, donor characteristics such as medical history, age, length of hospitalization, and cause of death have a significant impact on islet recovery after isolation [44]. It has been shown that brain death induces macrophage infiltration into islets and the augmentation of macrophages-associated inflammatory molecules such as TNF-α, IL-1β, and IL-6 in islets [45]. Proinflammatory cytokines such as IL-1β have a profound impact on pancreatic β-cell function and death during type 1 diabetes and pancreatic islet transplantation [46]. Moreover, they significantly reduce islet yield, viability, functionality, and engraftment after transplantation [42]. Therefore, it is important to develop therapeutic strategies to prevent/reduce proinflammatory cytokine release due to brain death and/or to block intracellular signaling associated with the cytokine.

In several clinical and experimental studies, males are more susceptible to the lethal effects of trauma, hemorrhage, and sepsis, compared to females in the proestrus cycle [47, 48]. Sex hormones such as 17β-estradiol contribute to this gender-specific immune response after trauma and severe blood loss [47]. Estradiol administration reverses the spontaneous increase in the expression and secretion of proinflammatory cytokines, such as TNF-α, IL-1, and IL-6 in humans after menopause [49]. Estradiol deficiency has been shown to enhance the responsiveness of cells toward some of these cytokines by upregulating cytokine receptor numbers and cofactors of cytokine action, thus amplifying the effects of increased cytokines [49]. In addition, estrogen possesses significant antioxidant and antiapoptotic activities [49, 50]. In the pancreas, functional estrogen receptors α and β have been demonstrated in both female and male pancreatic islets [51], and 17β-estradiol treatment after brain death significantly decreased proinflammatory cytokines [52].
and improved isolated human pancreatic islet survival after proinflammatory cytokine exposure by inhibition of JNK [53, 54]. These effects were associated with reduced JNK targets, including the nuclear activities of transcription factors AP-1, c-Jun, c-Fos, Jun-D and ATF-2, involved in apoptosis in β-cells [54]. The salutary effects of estradiol treatment were demonstrated by reduced islet apoptosis before islet isolation, increased islet yields and islet viability, and enhanced islet functionality in vitro and in vivo after transplantation, which will result in higher efficacy of islet transplantation. The application of treatment with 17β-estradiol to reduce the detrimental effects of brain death could improve the quality of organs from marginal donors, broadening the criteria for donor acceptance for islet isolation and transplantation.

During pancreas preservation, islets are subjected to severe adverse conditions that impair survival and ultimately contribute to graft failure after transplantation. University of Wisconsin (UW) solution is usually used for pancreas preservation. Recent reports have shown that the two-layer method (TLM), which employs oxygenated perfluorochemical (PFC) and UW solution, is superior to simple cold storage in UW solution [55–57]. Matsuda et al. showed caspase, JNK, and p38 activity in isolated islets after preservation of UW or TLM [58]. Caspase activation is mainly involved in islet apoptosis induced by simple UW storage, and it is blocked to a great extent by using TLM. Pancreas oxygenation by TLM contributes to the prevention of islet apoptosis by maintaining mitochondrial integrity and functions such as the production of ATP and phosphorylation of anti-apoptotic genes on the mitochondrial surface [59, 60]. On the other hand, activation of JNKS was strongly observed in both TLM and UW groups compared with the fresh group. Analyses of the intensity of phosphorylated JNKS showed a 2.5- and 4.0-fold increase in JNK activity in TLM and UW groups, respectively, compared with the fresh group. There was no difference in the activity of p38 among these groups. Although pancreas preservation with TLM before islet isolation has been demonstrated to improve islet yield, quality, and transplant results by protecting the activation of caspase, TLM preservation, as with simple UW preservation, cannot protect against JNK activation.

### Islet isolation and purification

During isolation and purification, islets are exposed to mechanical, enzymatic, osmotic, and ischemic stresses. Abdelli et al. mapped the major intracellular stress-signaling pathways that may mediate human islet loss during isolation and following cytokine attack [15]. The isolation procedure potently recruits two pathways, MKK7 → JNK/p38 → c-fos, and NF-κB → iNOS, indicating that islets sense the isolation procedure as an important stress recruiting the major proapoptotic intracellular pathways. Proinflammatory cytokines activate the NF-κB → iNOS and MKK4/ MKK3/6 → JNK/p38 pathways without recruitment of c-fos. It is also likely that the isolation procedure together with cytokines produced during the inflammatory process immediately following transplantation may further synergize to enhance cell death [61].

Pharmacological inhibition of the JNK and p38 pathways from the beginning of isolation and throughout the transplantation procedure might prove critical for the maintenance of islet cell mass and the lowering of primary nonfunction in an animal model [60, 62, 63]. We showed recently that delivery of a JNK inhibitory peptide via the protein transduction system can prevent apoptosis of isolated islets [60]. Protein transduction systems have been employed for the delivery of proteins/peptides, which are commonly unable to cross cell membranes, into many cell types [64–76]. A series of protein transduction domains (PTDs, also known as cell-penetrating peptides; CPPs), have been shown to cross biological membranes efficiently and to promote the delivery of peptides and proteins into cells. We synthesized JNK inhibitory peptide (JNKI) as a C-terminal fusion peptide with the 11-arginine PTD (11R-JNKI). 11R efficiently delivered the JNK inhibitory peptide into isolated islets and 11R-JNKI prevented islet apoptosis immediately after isolation and improved islet graft function. These findings suggest that inhibition of JNK activity could be useful to prevent the impairment of islet cells and lead to improved outcomes for pancreatic islet transplantation.

### Islet transplantation

Thrombosis and inflammation are linked in many clinical conditions including islet transplantation [77–79]. Proinflammatory mediators may regulate coagu-
lation activation and products of the clotting cascade may affect inflammation [79–81]. Although intraportal infusion of isolated islets is the preferred site for clinical islet transplantation, instant coagulation/inflammation reactions (IBMIR) [78, 82, 83] occur regularly during this procedure, even without clinical signs of intraportal thrombosis [77, 84]. IBMIR induces the production of proinflammatory cytokines, such as TNF-α, IL-1β, interferon-γ, and NO, and contributes to the disruption of islet morphology, islet dysfunction, and death, leading to early graft loss [84–86]. Inhibition of IBMIR, such as low molecular weight dextran sulfate (LMW-DS) and activated protein C (APC), would improve the outcome of clinical islet transplantation. The efficacy of LMW-DS in preventing xenogeneic [87] and allogeneic IBMIR in vitro and in vivo has been recently demonstrated [88] and the antithrombotic, profibrinolytic, anti-inflammatory, and antiapoptotic activities of APC decrease graft loss in the peritransplant period by decreasing IBMIR, apoptosis, and endothelial cell activation after intraportal islet transplantation [89].

Although the production of proinflammatory cytokines, such as TNF-α, IL-1β, and interferon-γ, has been demonstrated after islet transplantation, intracellular stress-signaling pathways such as JNK and p38 during the islet transplant process are not well-known. We recently mapped the JNK pathway that mediates islet loss during islet transplantation using a mouse model (Noguchi et al. unpublished data). JNK activity in the liver after islet transplantation became progressively higher at least until 24 hrs. This certainly has profound implications for the release of proinflammatory cytokine and thus islet apoptosis. Moreover, we showed that an intraportal injection of cell-permeable JNK inhibitor during islet transplantation can prevent islet graft loss immediately after transplantation. The activity of JNK maintained an extremely low level in the liver at least until 24 hrs after transplantation and islet transplant outcomes were improved (Noguchi et al. unpublished data). These data suggest that JNK inhibitory peptide prevents JNK activation immediately after islet transplantation in the liver and reduces JNK activity in insulin target organs, resulting in improved islet transplant outcomes.

**Conclusion**

Prevention of islet apoptosis due to several forms of stress before transplantation may yield a greater number of healthy functional islets per isolation. Prevention of islet apoptosis during islet transplantation also improves islet graft function. Pharmacological inhibition of the JNK pathways, such as 17β-estradiol and 11R-JNKI, from the beginning of isolation and throughout the transplantation procedure might prove critical for the maintenance of islet cell mass and the lowering of primary nonfunction. The control of intracellular signaling pathways including JNK is extremely important in islet transplantation.

**References**

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