Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 354 (2007) 227–233
BBRC
www.elsevier.com/locate/ybbrc

The L-isoform but not D-isoforms of a JNK inhibitory peptide protects pancreatic β-cells

Alessia Fornoni [a,b,*], Lorenzo Cobianchi [c], Nahir Y. Sanabria [a], Antonello Pileggi [a], R. Damaris Molano [a], Hirohito Ichii [a], Samuel Rosero [a], Luca Inverardi [a], Camillo Ricordi [a], Ricardo L. Pastori [a,*]

[a] *Diabetes Research Institute, University of Miami Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA*
[b] *Division of Nephrology and Hypertension, University of Miami Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA*
[c] *Istituto di Chirurgia Generale Epatopancreatica, Dipartimento di Scienze Chirurgiche, Universita' degli Studi di Pavia, IRCCS Policlinico 'San Matteo', Pavia, Italy*

Received 20 December 2006
Available online 2 January 2007

Abstract

The activation of c-jun N-terminal kinase (JNK) in pancreatic islets is associated with impaired function and viability, and JNK inhibitory peptides (JNKIs) are cytoprotective. In particular, L-isoforms of JNKIs were shown to improve islets viability, while the D-retro-inverso isoform of JNKI (RI-JNKI), with a higher therapeutic potential due to longer half-life, has not been studied. We compared the cytoprotective properties of L-JNKI and RI-JNKI. Treatment of murine islets with L-JNKI resulted in preservation of islet equivalents and greater percentage of viable β-cells in culture. In contrast, RI-JNKI was not protective. We found that L-JNKI but not RI-JNKI prevents endogenous c-jun phosphorylation in insulinoma cells. Moreover, RI-JNKI induced islet cells necrosis and activates the p-38 kinase. In conclusion, L-JNKI directly affects β-cells and ameliorates islet viability and function, while RI-JNKI has toxic effects, limiting its biological application to islet cell biology.
© 2007 Published by Elsevier Inc.

Keywords: Pancreatic islets; Cytoprotection; Inflammation; MAPK; JNK inhibition; Retroinverso peptide; D-Aminoacids; Diabetes; Islets transplantation

Protein transduction domain (PTD) technology is a novel approach that allows the direct intracellular delivery of small cell penetrating peptides that are able to cross the hydrophobic lipid bilayer of the plasma membrane. As a result, biologic modifiers (including proteins) attached to PTDs are shuffled inside the cell. The first and most commonly used PTD was derived from the basic sequence of the human immunodeficiency virus (HIV-1) transactivator TAT protein [1,2]. More recently, poly-arginine (polyR) has been shown to exhibit a great efficiency in terms of peptide delivery [3,4]. A strategy used to produce biologically active peptides resistant to proteases is to generate retroinverso (RI) peptides. The RI version of a L-peptide consists of a reverse peptide sequence with inverted chirality (substituting L aminoacids with D enantiomers) [5]. As a result, partial or complete RI modification of small peptides has been shown to retain tissue-trophic activity and to have a longer half-life [6]. In fact, treatment with either the L [2] or the RI [7] isoform of several PTDs has been successfully performed in vivo in several animal models and offers temporal and reversible regulation of a biological process.

Indeed, several biological applications of PTDs have been experimented in pancreatic islet cell biology. We found that anti-oxidative stress proteins such as Heme Oxygenase-1 [8] and anti-apoptotic proteins such as Bcl-XL [9] can be effectively delivered to human islets when coupled to a TAT-derived PTD and have significant biological effects. In addition, peptides that inhibit intracellular

* Corresponding authors. Fax: +1 305 243 4404.
E-mail addresses: afornoni@med.miami.edu (A. Fornoni), rpastori@med.miami.edu (R.L. Pastori).

0006-291X/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2006.12.186

kinases such as JNK [10,11] and IkB [12] have also been successfully used. JNKIs are more specific than the more commonly used ATP-competitive inhibitors, and lower concentrations might be required for effective competition to occur [13]. The most studied JNKI consists of a 20-amino acids sequence of the docking domain of the Islet/Brain-1protein (IB-1 protein) fused to the TAT-PTD [10], which has been shown to inhibit all three types of JNKs.

In the specific area of islet transplantation, pancreas preservation and islet cell processing and implantation are conditions of stress associated with the release of multiple pro-inflammatory cytokines leading to JNK activation [14–17]. In human pancreatic islets, JNK inhibition via an ATP-competitive agent (SP600125) has been shown to reduce caspase-3 activity [17]. The L-isoform of TAT-JNKI (L-JNKI) protects from IL-1β-induced cell death studied in the murine insulinoma cell line βTC-3 [10]. More recently, the L-isoform of an 11R-JNKI has been shown to transduce into isolated porcine islets and to prevent JNK activity [11]. Although both ATP-noncompetitive and ATP-competitive JNK inhibitors affect the viability of porcine and human islets, the biological activity of RI-JNKI or hybrid isoforms have not been tested in islet cells and will be the subject of the present study.

Research design and methods

Cell culture. The murine insulinoma cell line NIT-1 was used: cells were treated for 48 hours (h) with 10 or 25 μM JNKIs synthesized as previously described for the L (L-JNKI) and the retroinverso isoform (RI-JNKI) [10] (Sigma Genosis, St. Louis, MO). A partial D-modification of the JNKI linked to nine arginines was also tested (D9R-LJNKI). After 48-h, cells were exposed to IL-1β (R&D Systems, Minneapolis, MN) at a concentration of 50 U/ml for 30 min and cell lysates were collected (Bio-Rad, Hercules, CA). A similar concentration of SP600125 (Sigma, St. Louis, MO), an ATP-competitive JNK inhibitor, was used as internal control. Irrelevant peptides (TAT alone in either RI- or L-isoform) were tested in preliminary experiments and compared to untreated cells.

Assessment of c-jun, JNK, Ik-B, ERK and P-38 phosphorylation. An immunoassay using laser flow cytometry (BioPlex, Bio-Rad) was used to study intracellular signaling [18]. Cells and islets (~250 islet equivalents, IEQ) were collected in lysis buffer. After determination of protein concentration (DC, Bio-Rad), endogenous protein phopshorylation was determined with the Bioplex platform as per manufacturer recommendations. A protein concentration of 0.5 μg/ml was determined to be optimal based on preliminary experiments with serial dilution of protein samples in order to insure the linear relationship between protein concentration and fluorescence. Results are expressed as ratios of phosphorylated to total protein.

Islets isolation and culture. Adult porcine islets were isolated and cultured using a modification of the automated method, and purified by sedimentation on density gradients [19,20]. For murine islets, C57BL/6 mice (B6; Jackson Laboratories, Bar Harbor, Maine) manipulations were conducted under protocols approved by the Institutional Animal Care and Use Committee (IACUC). The pancreata were digested with collagenase Type V retrogradely infused through the pancreatic duct, and islets isolated on density gradients and cultured as previously described [21].

Assessment of islet equivalents. Freshly isolated islets were cultured either under standard conditions or in the presence of 10 μM JNKIs. Islets were counted before and after overnight culture with diphenylthiocarbazone (DTZ) staining, IEQ calculated as previously described [22,23], and data expressed as percentage of islet loss.

Assessment of β-cell viability and mitochondrial membrane potential. β-Cells viability was assessed as previously described [24]. Briefly, islets were pretreated for 48 h with L-JNKI (10 μM). A cocktail of cytokines (CTK: IL-1β 50 U/ml, TNF-α 1000 U/ml and INF-γ 1000 U/ml) was added 18 h prior to islets dissociation. Single cell suspensions were incubated with 1 μM Newport Green (NG) and stained with 7-aminoactinomycin D (7AAD, Invitrogen, Carlsbad, CA). The zinc-binding dye Newport Green (NG) allows identification and quantification of β-cells on dissociated islets, based on the abundant zinc content in β-cell secretory granules [25]. The membrane exclusion dye 7-aminoactinomycin D (7AAD, Invitrogen, Carlsbad, CA) was used as marker of cell death. Islet β-cells viability was determined as NG+/7AAD− cells. For selected experiments, 100 ng/ml of tetramethylrhodamine ethyl ester (TMRE, Molecular Probes, Eugene, OR) was added and used for the assessment of mitochondrial membrane potential. Data were expressed as TMRE+ cells on gated NG+ 7AAD− cells (viable β-cells). This method allows for the detection of early apoptosis and is predictive of islet function *in vivo* [24].

Assessment of cell necrosis and apoptosis. NIT-1 cells or islet single cell suspensions were stained with a fluorescein isothiocyanate (FITC)-labeled analog of the pan-caspase inhibitor Z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[*O*-methyl]-fluoromethylketone) in which a carbobenzoxy (Z) N-terminal blocking group was substituted to create the fluorescent apoptosis marker (CaspACE™ FITC-VAD-FMK; Promega Corporation, Madison, WI) [9]. This compound binds irreversibly to intracellular activated caspases allowing for the detection of apoptotic cells. Samples were analyzed by flow cytometry (FACSCalibur, Becton–Dickinson, Mountain View, CA).

Data analysis. Results represent the mean of 3–5 independent experiments performed in duplicate or triplicate. Results are expressed as means ± SD. Results were compared using analysis of variance (ANOVA). When ANOVA showed a statistically significant difference, a group-by-group comparison was performed using a *t*-test with a Tukey's correction for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

L-JNKI but not RI-JNKI preserved islet equivalents in culture

We compared different JNK inhibitory peptides as described in Table 1. We tested whether RI-JNKI (an isoform that has both the sequence of the PTD and the JNK inhibitor reversed with D-aminoacids) may have a similar effect to that observed for L11R-JNKI in the preservation of islet mass *in vitro* [11]. Islets were treated immediately after isolation with either L-JNKI (were both the sequence of the TAT PTD and of the JNK inhibitor were in the L-isoform) or RI-JNKI (10 μM). The percentage of IEQ loss was reduced from 48% (control) to 34% (L-JNKI treated, Fig. 1A). In order to understand whether β-cells were protected, we analyzed the percentage of NG+ cells (β-cells) [24,25]. Treatment with L-JNKI immediately after isolation and for the subsequent 48 h led to an increase in the number of NG+/7AAD− cells (viable β-cells). This effect was not observed when RI-JNKI was used (Fig. 1B).

L-JNKI protects β-cells from cytokine-induced cell death

In order to understand if RI-JNK would be effective under stress conditions, we tested the hypothesis that L-JNKI but not RI-JNKI could inhibit the loss of viable β-cells induced by the treatment with a cytokines cocktail (CTK). We found that islet pretreatment with a L-JNKI prior to CTK exposure almost completely prevented β-cell

Table 1
List and sequence of peptides used

Short	Peptide	Sequence
L-TAT	L-TAT alone	GRKKRRQRRRPP
RI-TAT	Retroinverso TAT alone	Pprrrqrrkkrg
L-JNKI	L-TAT + JNK inhibitor	GRKKRRQRRRPPRPKRPTTLNLFPQVPRSQDT
RI-JNKI	Retroinverso TAT + JNK inhibitor	tdqsrpvqpflnlttprkprpprrrqrrkkrg
D9R-JNKI	D9 arginines + L-JNK inhibitor	rrrrrrrrrggRPKRPTTLNLFPQVPRSQDT

Short name used for each peptide (first column); description of each peptide (second column), aminoacidic sequence (third columns). L-Aminoacids are in capitalized letters, D-aminoacids in lowercase letters.

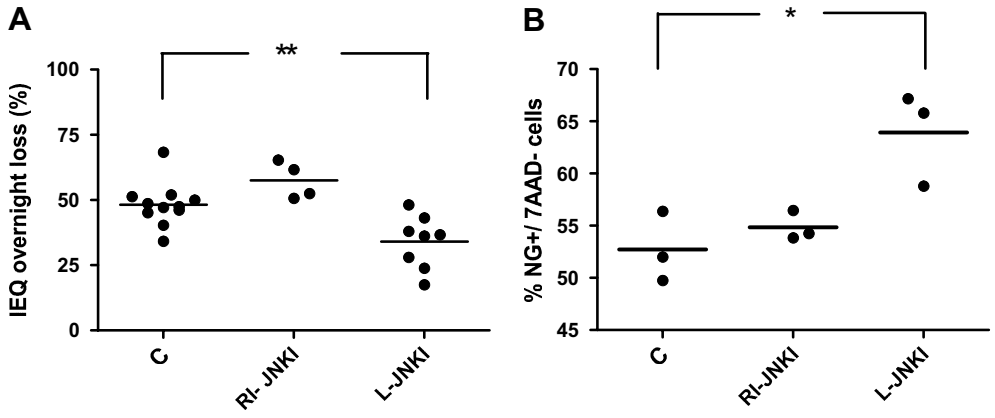

Fig. 1. Effect of L-JNKI on islet equivalents and on percentage of viable β-cells. Untreated murine islets were compared to islets treated immediately after isolation with either RI-JNKI or L-JNKI (10 μM). Islet equivalent count was performed after 12–18 h of culture (A), and data expressed as percentage loss observed in JNKI treated vs control. The percentage of β-cells in the alive population (NG+/7AAD−) is shown in (B). $^{*}p < 0.05$.

death (Fig. 2A). However, pretreatment of islets with RI-JNKI was not protective from cytokines-induced cell death and altered the mitochondrial membrane potential itself (Fig. 2B).

L-JNKI but not RI-JNKI prevents IL-1β-induced phosphorylation of endogenous c-jun in NIT-1 cells

In order to understand whether the lack of activity of RI-JNKI in islet cells was related to its inability to down-regulate endogenous c-jun phosphorylation, NIT-1 cells were pretreated for 48 h with several peptides described in Table 1. Cells were then exposed to IL-1β (50 U/ml) for 30 min and endogenous c-jun phosphorylation was evaluated to assess the potency of each peptide (Fig. 3). The inhibition achieved with L-JNKI was similar to the one observed with SP600125, an ATP-competitive JNK inhibitor. The effect of L-JNKI was significant at 10 μM; increasing the dose to 25 μM did not further suppress endogenous c-jun. RI-JNKI and D9R-LJNKI (a D–L hybrid peptide consisting of 9D-arginine residues linked to the L-isoform of the JNK inhibitor), did not show any activity.

RI-JNKI and D9R-LJNKI induce necrosis of NIT-1 cells and porcine islets

A possible reason for the lack of function of RI-JNKI in islet cells is its potential toxicity. We performed flow cytometry analysis using 7-AAD as a marker of cell necrosis and FITC-VAD-FMK as a marker of apoptosis. NIT-1 cells were treated with different JNKIs or with SP600125. Both RI-JNKI and D9R-LJNKI induced a marked increase of the percentage of 7AAD+ cells, while L-JNKI and SP600125 did not (Fig. 4A and B), suggesting that D-aminoacids are toxic in cultured islet cells. None of the compound tested induced a significant activation of apoptosis (Fig. 4A). Furthermore, treatment with an irrelevant RI-TAT peptide at the same dose of 10 μM affected cell viability up to similar levels (data not shown). To exclude the possibility that the observed effect was occurring solely in cultured insulinoma cells, we repeated the same experiment using porcine islets. Any of the peptide containing D-aminoacids at 10 μM (RI-JNKI, D9R-LJNKI, RI-TAT) was found to induce necrosis in dissociated pig islets (Fig. 4C).

RI-JNKI and D9R-LJNKI paradoxically activates selective stress kinases

In order to understand the possible mechanisms of toxicity of RI-JNKI and D9R-LJNKI, we tested whether they could paradoxically stimulate JNK, p-38, ERK or Ik-B. We were able to demonstrate that both RI-JNKI and D9R-JNKI significantly affected p-38 phosphorylation when compared to L-JNKI (Fig. 5A). In addition, D9R-JNKI led also to a paradoxical activation of JNK phosphorylation (Fig. 5B). The toxicity of either compound was not mediated by NF-kB or ERK, since Ik-B and

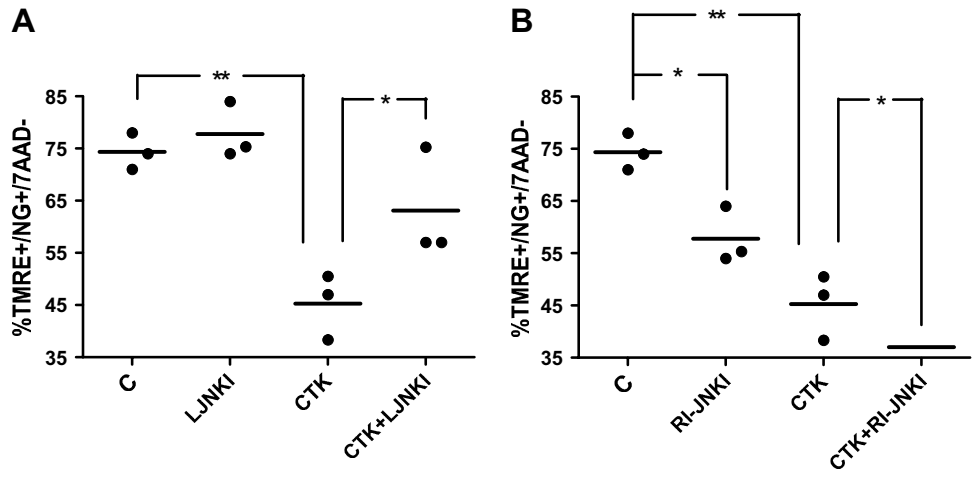

Fig. 2. Effect of CTK and L-JNKI on mitochondrial membrane potential. Untreated murine islets were compared to islets treated immediately after isolation with L-JNKI or RI-JNKI (10 μM). A cytokines cocktail (CTK: IL-1β 50 U/ml, TNF-α 1000 U/ml and INF-γ 1000 U/ml) was added to islet in culture 18 h prior to collection and analysis of %TMRE+/NG+/7AAD− in L-JNKI (A) or RI-JNKI (B) treated islets. $^{*}p < 0.05$; $^{**}p < 0.01$.

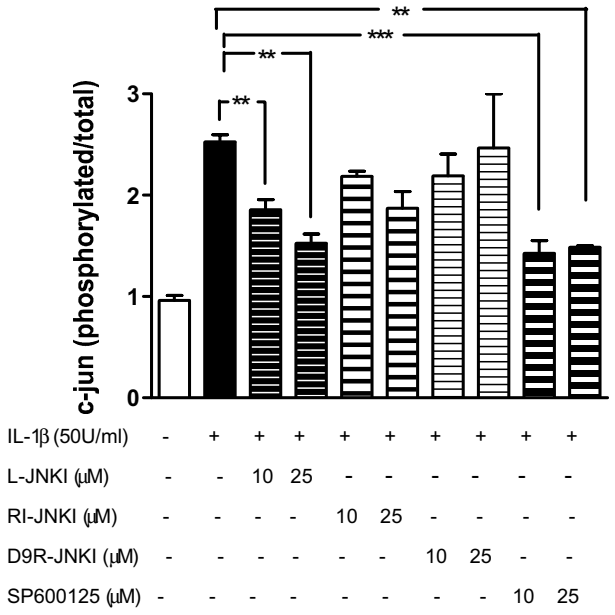

Fig. 3. Activity of several JNKIs on endogenous c-jun phosphorylation. NIT-1 cells where pretreated with several JNKIs for 48 h (10 or 25 μM), followed by treatment with IL-1β (50 U/ml) for 30 min and determination of endogenous c-jun phosphorylation. $^{**}p < 0.01$; $^{***}p < 0.001$.

ERK phosphorylation remained unaffected (data not shown).

Discussion

An increasing number of PTDs carrying bioactive molecules or inhibitors are being shown to have biological effects and therapeutic potential. While this may allow for clinical applications in a wide variety of settings, it is critical to validate their efficacy and safety for specific applications and cellular targets. JNKI have been partially tested in islet cells [10,11]. In particular an L-isoform of JNKI linked to a poly-R PTD has been shown to preserve porcine islet mass [11]. There has also been interest in a retroinverso-isoform of JNKI (RI-JNKI) [10], as this might have superior efficacy and clinical applicability because of its longer half-life. However, when using D-enantiomers, a concern related to their potential toxicity arises, since accumulation of D-amino acids occurs in senescence and characterizes certain degenerative disease such as Alzheimer dementia [26]. In addition, very high doses of RI-JNKI are needed to achieve function, which might enhance potential toxicity [10].

In this study we investigated the efficacy and safety of RI-JNKI in pancreatic islet cells. While L-JNKI showed the expected protective effects, RI-JNKI was not able to improve islet mass (Fig. 1A). In particular, and consistent with what was known in porcine islets treated with the L-isoform of a JNK inhibitory peptide linked to poly-R [11], we found a significant reduction in the percentage of islet equivalent loss in culture after isolation when islets were treated with L-JNKI but not with RI-JNKI. Similarly, in the population of viable islet cells (7AAD−), we were able to show that treatment with L-JNKI, but not RI-JNKI, increased the percentage of β-cells (Fig. 1A).

To test the hypothesis that JNKI protects β-cells even under condition of stress, we pretreated islets with L-JNKI and exposed them to a cocktail of proinflammatory cytokines (CTK: IL-1β, TNF-α, INF-γ) [27,28]. We then evaluated the mitochondrial membrane potential of β-cells, a feature of apoptotic and necrotic β-cell damage [24]. We found that L-JNKI protected from CTK-induced β-cells death (Fig. 2A). However, RI-JNK did not show such an effect and could induce a dissipation of the mitochondrial membrane potential itself (Fig. 2B).

We found a correlation between the lack of protection against cytokine-induced cell death by RI-JNKI and its inability to prevent phosphorylation of endogenous c-jun

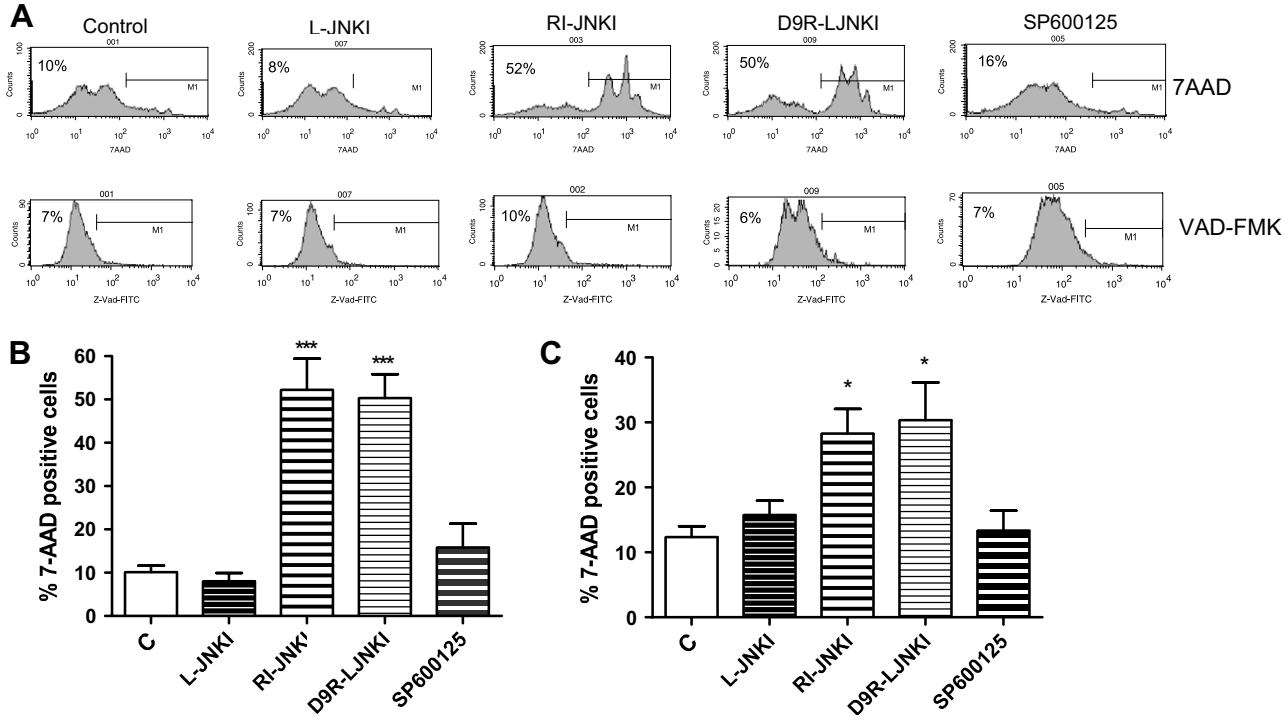

Fig. 4. Effect of RI-JNKI and D9R-LJNKI on NIT-1 cells and porcine islet cells necrosis and apoptosis. NIT-1 cells and porcine islets were treated with any of the JNKIs (10 μM). (A) Representative flow cytometry study for markers of cell necrosis (7AAD) and apoptosis (VAD-FMK). (B,C) Bar graph representation of five independent experiments of the percentage of 7AAD positive NIT-1 cells (B) and dissociated porcine islets (C) treated with different JNKIs. $^{*}p < 0.05$; $^{***}p < 0.001$.

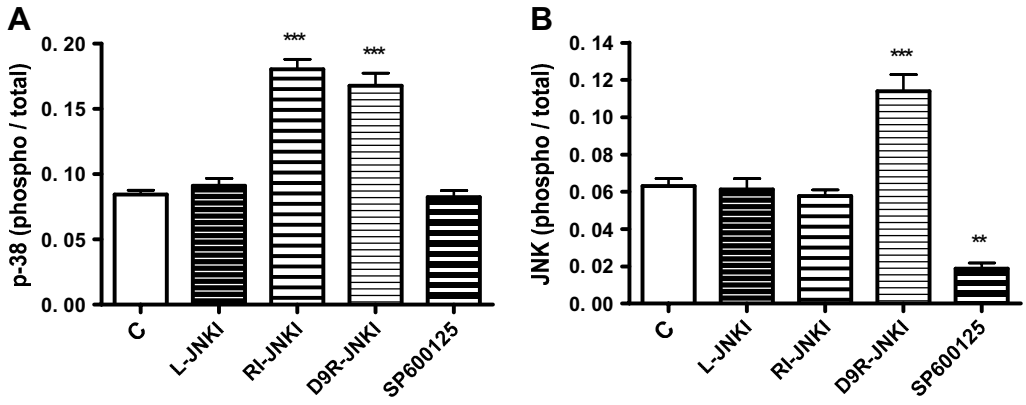

Fig. 5. RI-JNKI and D9R-LJNKI affect the phosphorylation of selected stress activated kinases. NIT-1 cells were treated with any of the JNKIs (10 μM), and cell lysates collected after 48 h for analysis. Both RI-JNKI and D9R-LJNKI significantly affected p-38 phosphorylation (A). In addition, D9R-LJNKI induced a paradoxical phosphorylation of JNK (B). $^{**}p < 0.01$; $^{***}p < 0.001$.

in NIT-1 cells. While L-JNKI had a dose dependent effect on c-jun phosphorylation similar to what is observed with a more standard ATP-competitive JNK inhibitor (SP600125), neither RI-JNKI nor D9R-LJNKI had any effect at any of the concentration tested. This data suggest that the presence of D-aminoacids residues in the JNK inhibitory peptides is responsible for the lack of activity on endogenous c-jun phosphorylation.

Since we did not find any effect of RI-JNKI in islet cells, either dependent or independent of c-jun phosphorylation, we tested the hypothesis that RI-JNKI is toxic to islet cells. We showed that both RI-JNKI or D9R-LJNKI induce cell necrosis in both NIT-1 cells and freshly isolated porcine islets. The possibility that poly-R per se is toxic to islet cells can be excluded because others have found that L-JNKI fused to L-11R protected islets in culture [11]. Thus, the presence of D-aminoacids, rather than retroinverso modification of the amino acid sequence, is responsible for the toxicity associated with the D-isoforms. Interestingly, the effect on cell death was not mediated by apoptosis, since neither RI-JNKI nor D9R-LJNKI induced caspase activation. Of remark, peptides with D-aminoacids were able to phosphor-

ylate stress activated kinases such as p-38, which could explain the mechanisms of D-aminoacids induced cell death, since the activity of p-38 is detrimental to islet viability [29]. Although we also observed a paradoxical activation of JNK by D9R-LJNKI, this most likely does not represent the mechanism of toxicity of D-aminoacids because JNK was not activated by RI-JNKI, which also was able to induce cell necrosis.

In conclusion, we have demonstrated that JNK inhibitory peptides containing D-amino acids do not exert a protective biological function in islet cells. This is most likely due to their toxicity, which results in direct cell necrosis. Our findings pose a major limitation to the application of D-aminoacids containing peptides in islet cell biology.

Acknowledgments

We acknowledge Dagmar Klein for her precious help and for her technical expertise. We thank Alberto Pugliese for careful review of the manuscript. We acknowledge the valuable assistance from JDRF-supported Preclinical Cell Processing and Imaging Cores. The current study was supported by grants DK-59993 (R.L.P.) from the National Institute of Health, a pilot project (A.F.) of a JDRF center Grant 4-2004-361 (C.R.), and by the Diabetes Research Institute Foundation.

References

[1] A.D. Frankel, C.O. Pabo, Cellular uptake of the tat protein from human immunodeficiency virus, Cell 55 (1988) 1189–1193.
[2] S.R. Schwarze, A. Ho, A. Vocero-Akbani, S.F. Dowdy, In vivo protein transduction: delivery of a biologically active protein into the mouse, Science 285 (1999) 1569–1572.
[3] P.A. Wender, D.J. Mitchell, K. Pattabiraman, E.T. Pelkey, L. Steinman, J.B. Rothbard, The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters, Proc. Natl. Acad. Sci. USA 97 (2000) 13003–13008.
[4] S. Futaki, T. Suzuki, W. Ohashi, T. Yagami, S. Tanaka, K. Ueda, Y. Sugiura, Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery, J. Biol. Chem. 276 (2001) 5836–5840.
[5] M. Chorev, R. Shavitz, M. Goodman, S. Minick, R. Guillemin, Partially modified retro-inverso-enkephalinamides: topochemical long-acting analogs in vitro and in vivo, Science 204 (1979) 1210–1212.
[6] E.M. Taylor, D.A. Otero, W.A. Banks, J.S. O'Brien, Retro-inverso prosaptide peptides retain bioactivity, are stable In vivo, and are blood–brain barrier permeable, J. Pharmacol. Exp. Ther. 295 (2000) 190–194.
[7] T. Borsello, P.G. Clarke, L. Hirt, A. Vercelli, M. Repici, D.F. Schorderet, J. Bogousslavsky, C. Bonny, A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia, Nat. Med. 9 (2003) 1180–1186.
[8] M.M. Ribeiro, D. Klein, A. Pileggi, R.D. Molano, C. Fraker, C. Ricordi, L. Inverardi, R.L. Pastori, Heme oxygenase-1 fused to a TAT peptide transduces and protects pancreatic beta-cells, Biochem. Biophys. Res. Commun. 305 (2003) 876–881.
[9] D. Klein, M.M. Ribeiro, V. Mendoza, S. Jayaraman, N.S. Kenyon, A. Pileggi, R.D. Molano, L. Inverardi, C. Ricordi, R.L. Pastori, Delivery of Bcl-XL or its BH4 domain by protein transduction inhibits apoptosis in human islets, Biochem. Biophys. Res. Commun. 323 (2004) 473–478.
[10] C. Bonny, A. Oberson, S. Negri, C. Sauser, D.F. Schorderet, Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death, Diabetes 50 (2001) 77–82.
[11] H. Noguchi, Y. Nakai, S. Matsumoto, M. Kawaguchi, M. Ueda, T. Okitsu, Y. Iwanaga, Y. Yonekawa, H. Nagata, K. Minami, Y. Masui, S. Futaki, K. Tanaka, Cell permeable peptide of JNK inhibitor prevents islet apoptosis immediately after isolation and improves islet graft function, Am. J. Transplant. 5 (2005) 1848–1855.
[12] K.K. Rehman, S. Bertera, R. Bottino, A.N. Balamurugan, J.C. Mai, Z. Mi, M. Trucco, P.D. Robbins, Protection of islets by in situ peptide-mediated transduction of the Ikappa B kinase inhibitor Nemo-binding domain peptide, J. Biol. Chem. 278 (2003) 9862–9868.
[13] M.A. Bogoyevitch, Therapeutic promise of JNK ATP-noncompetitive inhibitors, Trends. Mol. Med. 11 (2005) 232–239.
[14] S. Abdelli, J. Ansite, R. Roduit, T. Borsello, I. Matsumoto, T. Sawada, N. Allaman-Pillet, H. Henry, J.S. Beckmann, B.J. Hering, C. Bonny, Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure, Diabetes 53 (2004) 2815–2823.
[15] A. Ammendrup, A. Maillard, K. Nielsen, N. Aabenhus Andersen, P. Serup, O. Dragsbaek Madsen, T. Mandrup-Poulsen, C. Bonny, The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic beta-cells, Diabetes 49 (2000) 1468–1476.
[16] L. Rosenberg, R. Wang, S. Paraskevas, D. Maysinger, Structural and functional changes resulting from islet isolation lead to islet cell death, Surgery 126 (1999) 393–398.
[17] R. Aikin, D. Maysinger, L. Rosenberg, Cross-talk between phosphatidylinositol 3-kinase/AKT and c-jun NH2-terminal kinase mediates survival of isolated human islets, Endocrinology 145 (2004) 4522–4531.
[18] S.M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, X. Zhang, P. Vincent, M. McHugh, Y. Cao, J. Shujath, S. Gawlak, D. Eveleigh, B. Rowley, L. Liu, L. Adnane, M. Lynch, D. Auclair, I. Taylor, R. Gedrich, A. Voznesensky, B. Riedl, L.E. Post, G. Bollag, P.A. Trail, BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, Cancer Res. 64 (2004) 7099–7109.
[19] H. Ichii, A. Pileggi, R.D. Molano, D.A. Baidal, A. Khan, Y. Kuroda, L. Inverardi, J.A. Goss, R. Alejandro, C. Ricordi, Rescue purification maximizes the use of human islet preparations for transplantation, Am. J. Transplant. 5 (2005) 21–30.
[20] C. Ricordi, C. Socci, A.M. Davalli, C. Staudacher, P. Baro, A. Vertova, I. Sassi, F. Gavazzi, G. Pozza, V. Di Carlo, Isolation of the elusive pig islet, Surgery 107 (1990) 688–694.
[21] A. Pileggi, R.D. Molano, T. Berney, H. Ichii, S. San Jose, E. Zahr, R. Poggioli, E. Linetsky, C. Ricordi, L. Inverardi, Prolonged allogeneic islet graft survival by protoporphyrins, Cell Transplant. 14 (2005) 85–96.
[22] Z.A. Latif, J. Noel, R. Alejandro, A simple method of staining fresh and cultured islets, Transplantation 45 (1988) 827–830.
[23] C. Ricordi, D.W. Gray, B.J. Hering, D.B. Kaufman, G.L. Warnock, N.M. Kneteman, S.P. Lake, N.J. London, C. Socci, R. Alejandro, et al., Islet isolation assessment in man and large animals, Acta Diabetol. Lat. 27 (1990) 185–195.
[24] H. Ichii, L. Inverardi, A. Pileggi, R.D. Molano, O. Cabrera, A. Caicedo, S. Messinger, Y. Kuroda, P.O. Berggren, C. Ricordi, A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations, Am. J. Transplant. 5 (2005) 1635–1645.
[25] B. Lukowiak, B. Vandewalle, R. Riachy, J. Kerr-Conte, V. Gmyr, S. Belaich, J. Lefebvre, F. Pattou, Identification and purification of functional human beta-cells by a new specific zinc-fluorescent probe, J. Histochem. Cytochem. 49 (2001) 519–528.

[26] S.A. Fuchs, R. Berger, L.W. Klomp, T.J. de Koning, D-amino acids in the central nervous system in health and disease, Mol. Genet. Metab. 85 (2005) 168–180.
[27] B. Kutlu, A.K. Cardozo, M.I. Darville, M. Kruhoffer, N. Magnusson, T. Orntoft, D.L. Eizirik, Discovery of gene networks regulating cytokine-induced dysfunction and apoptosis in insulin-producing INS-1 cells, Diabetes 52 (2003) 2701–2719.
[28] D.L. Eizirik, T. Mandrup-Poulsen, A choice of death–the signal-transduction of immune-mediated beta-cell apoptosis, Diabetologia 44 (2001) 2115–2133.
[29] T. Matsuda, K. Omori, T. Vuong, M. Pascual, L. Valiente, K. Ferreri, I. Todorov, Y. Kuroda, C.V. Smith, F. Kandeel, Y. Mullen, Inhibition of p38 pathway suppresses human islet production of pro-inflammatory cytokines and improves islet graft function, Am. J. Transplant. 5 (2005) 484–493.