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Research Article

The imidazoline RX871024 induces death of proliferating insulin-secreting cells by activation of c-jun N-terminal kinase

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Abstract. An insufficient number of insulin-producing β-cells is a major cause of defective control of blood glucose in both type 1 and type 2 diabetes. The aim of this study was to clarify whether the insulinotropic imidazolines can affect the survival of highly proliferating insulin-secreting cells, here exemplified by the MIN6 cell line. Our data demonstrate that RX871024, but not efaroxan, triggered MIN6 cell death and potentiated death induced by a combination of the pro-inflammatory cytokines interleukin-1β, interferon-γ and tumor necrosis factor-α. These effects did not involve changes in nitric oxide production but correlated with stimulation of c-jun N-terminal kinase (JNK) activity and activation of caspases-1, -3, -8 and -9. Our results suggest that the imidazoline RX871024 causes death of highly proliferating insulin-secreting cells, putatively *via* augmentation of JNK activity, a finding that may impact on the possibility of using compounds of similar activity in the treatment of diabetes.

Keywords. Apoptosis, insulin-secreting cell, cytokine, imidazoline, MAPK, caspase.

Introduction

Type 1 diabetes is an autoimmune disease characterized by a selective destruction of insulin-secreting pancreatic β-cells due to inflammation in the islets of Langerhans [1]. Pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), secreted by macrophages and T lymphocytes infiltrating the pancreatic islets contribute to the development of type 1 diabetes by acting directly on the β-cells [2–4]. Pro-inflammatory cytokines induce β-cell death when added in combination [5]. Cytokine-mediated signal transduction in β-cells involves activation of mitogen-activated protein kinases (MAPK), including c-jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK) [6]. We have previously shown that MAPK activation participates in β-cell apoptosis [7, 8], partly through induction of nitric oxide (NO) production. Cytokine-induced expression of inducible NO synthase (iNOS) and an increase in intracellular NO concentration is associated with β-cell death [9, 10]. Previously, we and others have demonstrated the importance of activating the effector caspase-3 for cytokine-induced triggering of apoptosis in pancreatic islets and in insulin-secreting cell lines [11, 12]. Caspase-3 is activated by initiator caspases [13, 14]

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such as caspase-1, caspase-8 and caspase-9, known to be activated by cytokines in pancreatic β-cells.

In case of type 2 diabetes, β-cell mass is diminished due to increased apoptosis [15] leading to insulin deficiency and overt hyperglycemia. We have demonstrated previously that one of the reasons for β-cell death in the course of type 2 diabetes is β-cell apoptosis caused by chronic hyperglycemia [16] and dependent upon caspase-3 activation [11].

A number of imidazoline compounds have been shown to promote insulin secretion and, due to this property, were proposed as potential agents for therapeutic treatment of type 2 diabetes mellitus [17–20]. In addition, we have previously shown that the imidazoline compound RX871024 does not induce apoptosis in primary pancreatic β-cells, but on the contrary even protects against IL-1β-induced primary β-cell apoptosis [10]. This effect was accompanied by inhibition of IL-1β-induced expression of iNOS. Although, a protective effect of RX871024 on primary β-cell apoptosis disappeared when a combination of the pro-inflammatory cytokines IL-1β, TNF-α and IFN-γ was used, this imidazoline did not increase cytotoxicity under these conditions [21].

Albeit low, the pancreatic β-cell has a certain proliferative capacity, which may actually be induced under certain conditions [22–24]. With this in mind any treatment of diabetes should never compromise this proliferative capacity. We used the insulin-secreting cell line MIN6 to help clarify the extent to which imidazoline compound RX871024 affects survival of proliferating insulin-secreting cells.

Materials and methods

Materials. DMEM and fetal calf serum were obtained from Gibco (Middlesex, UK). RX871024 was obtained from Reckitt and Colman (UK). Efaroxan and recombinant murine TNF-α were purchased from Sigma (St. Louis, MO, USA), recombinant human IL-1β from Calbiochem (San Diego, CA, USA), recombinant murine IFN-γ from Life Technologies (Gaithersburg, MD, USA), and reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from Invitrogen (Carlsbad, CA, USA). The JNK substrate, GST-c-jun [1–79] was from Calbiochem and recombinant murine heat shock protein-25 (Hsp25) from Stressgen (Victoria, Canada). GST-Elk-1 was synthesized by standard glutathione-S-transferase (GST) fusion protein expression and purification procedures using the RediPack GST Purification Module kit (Amersham Biosciences, Buckinghamshire, UK). [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham. All other reagents were of analytical grade and were obtained from VWR International (West Chester, PA, USA).

Cell culture. The β-cell line MIN6 (passages 32–42) was cultured in DMEM containing 25 mM glucose supplemented with 10 % (vol/vol) fetal calf serum, 50 μM β-mercaptoethanol, 50 U/ml penicillin, and 50 μg/ml streptomycin as previously described [25]. MIN6 cells were exposed to imidazolines with or without a combination of cytokines (25 U/ml IL-1β, 100 U/ml IFN-γ, 100 U/ml TNF-α) for 20 h and lactate dehydrogenase (LDH) release, nitrite production and activity of caspases-1, -3, -8 and -9 were measured.

Assessment of MIN6 cell death. Assessment was performed using CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI, USA), according to the manufacturer's specifications. The method is based on quantitative measurements of LDH upon cell lysis. Absorbance was recorded at 490 nm with a 96-well plate reader. Cell death was assessed as percentage of LDH released into the culture medium in relation to the total LDH content.

MIN6 cell nitrite production. Nitrite production was determined using the Griess reaction. Culture medium was withdrawn and centrifuged for 2 min at 1500 *g* and 100-μl samples of supernatant were transferred to a 96-well plate and mixed with 50 μl Griess reagent (Alexis Corporation, Carlsbad, CA, USA), as previously described [10]. The reaction was carried out for 15 min at room temperature. Nitrite production was determined by measuring absorbance at 540 nm with reference at 620 nm in a 96-well plate reader. For calibration of data, a standard curve for $NaNO_2$ in DMEM medium was established in each assay. The results are expressed as μM NO_2^-/μg protein.

***In vitro* kinase assay.** Phosphotransferase activities toward GST-c-jun, GST-Elk-1 and Hsp25 were measured by a whole-cell lysate *in vitro* kinase assay as previously described [21]. Phosphorylated substrates were visualized by autoradiography and quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA, USA).

Caspase activity measurement. Caspase activity was measured using fluorometric Caspase-3 and Caspase-8 Activity Assays (Oncogene, Darmstadt, Germany), and Caspase-1 and Caspase-9 Fluorometric Assays (R&D Systems, Minneapolis, MN, USA) according to the supplier's instructions. The assays are based on the cleavage of a caspase-specific substrate labeled with a fluorescent molecule, 7-amino-4-trifluoromethyl coumarin (AFC). Reaction was monitored by a blue to

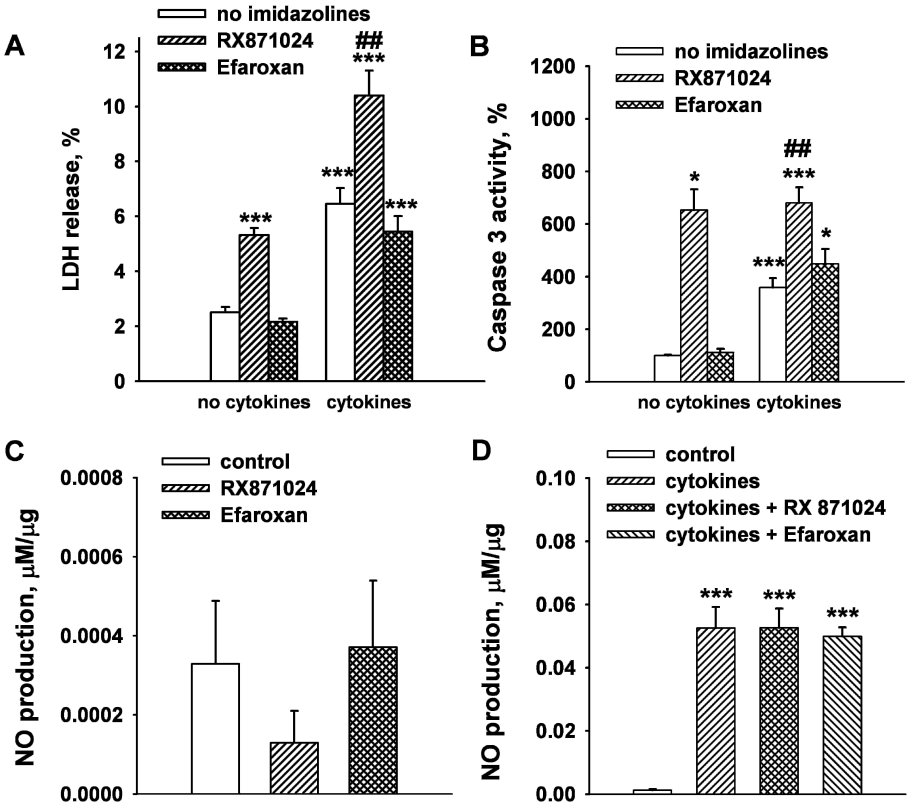

Figure 1. Effect of RX871024 and efaroxan on death, NO formation and caspase-3 activity in MIN6 cells. Cells were incubated in culture medium in the presence or absence of a combination of cytokines (IL-1β + IFN-γ + TNF-α) with or without 50 μM RX871024 or 50 μM efaroxan for 20 h. (*A*) Cell death was assessed by estimating lactate dehydrogenase (LDH) release. (*B*) Caspase-3 activity was measured by a fluorometric assay. (*C*, *D*) NO production was measured as NO_2^- release into the medium using Griess reagent. Results are means ± SE (n=3–6). Statistical significance between means was assessed by Student's t-test for unpaired values. $^{*}p<0.05$ vs control; $^{***}p<0.0005$ vs control; $^{\#\#}p<0.005$ vs cytokines.

green shift in fluorescence upon cleavage of the AFC fluorophore. In brief, 50 μl cell lysate was mixed with 50 μl reaction buffer and caspase fluorogenic substrate. After incubation at 37 °C for 2 h, readings were performed using a fluorescence plate reader, with excitation and emission wavelengths of 400 nm and 505 nm, respectively, and a longpass filter of 430 nm, which transmits wavelengths longer than 430 nm and blocks shorter wavelengths. Results were quantified as relative fluorescence units of caspase activity per μg of protein and expressed as a percentage of control.

Presentation of results. Data analysis was performed using Sigma Plot 2001 for Windows (Jandel Corp., USA). All results are expressed as means ± SE for the indicated number of experiments.

Results

RX871024, but not efaroxan, causes MIN6 cell death in both the absence and presence of cytokines. We previously discovered that the imidazoline compound RX871024 does not induce apoptosis in primary pancreatic β-cells and even protects against IL-1β-induced primary β-cell apoptosis [10]. To clarify the extent to which RX871024 affects survival of proliferating insulin-secreting cells we have investigated the effects of the imidazoline on MIN6 cell death and compared these effects with those of efaroxan, as a reference insulinotropic imidazoline [26, 27]. MIN6 cells were incubated in the absence or presence of a mixture of cytokines with or without addition of 50 μM RX871024 or efaroxan. RX871024 and efaroxan at this concentration have been shown to potently induce insulin release [17, 21, 28]. After incubation, cell death was detected by estimation of percentage of LDH released into the culture medium. As expected, cytokines induced MIN6 cell death (Fig. 1A). Unlike the effect of RX871024 on primary β-cells [10, 21], RX871024 caused a substantial elevation in MIN6 cell death at 50 μM in both the absence and presence of cytokines. In contrast, efaroxan did not affect MIN6 cell death regardless the presence of cytokines. These observations indicate that RX871024 potentially can induce death of proliferating insulin-secreting cells.

Neither RX871024 nor efaroxan affect cytokine-induced NO formation in MIN6 cells. We have previously shown that RX871024 protects against IL-1β-induced apoptosis in mouse β-cells by inhibiting IL-1β-induced synthesis of NO [10]. To investigate the mechanisms underlying the cytotoxic effect of RX871024 on MIN6 cells, we examined how the imidazoline affects NO formation induced by pro-inflammatory cytokines. The cells were incubated in

the absence or presence of the combination of cytokines with or without addition of 50 μM RX871024 or efaroxan and nitrite concentration in the culture medium was evaluated with the Griess reaction. However, in contrast to our previous report using primary β-cells [21], neither of the imidazolines affected NO formation in insulinoma MIN6 cells in the absence or presence of cytokines (Fig. 1C, D), suggesting that changes in NO production cannot be responsible for the cytotoxic effect of RX871024 on MIN6 cells.

RX871024 stimulates activation of JNK in MIN6 cells. MAPKs are essential mediators of cytokine effects in β-cells leading to iNOS expression and apoptosis [7, 8, 29]. We therefore examined the effect of RX871024 or efaroxan on cytokine-induced signal transduction at the level of MAPKs. MIN6 cells were incubated in the absence or presence of the mixture of cytokines with or without addition of 50 μM RX871024 or efaroxan for 1 h and cell lysates were subjected to *in vitro* kinase assay using the substrates Elk-1, c-jun and Hsp25. Elk-1 is a substrate of ERK, c-jun a substrate of JNK, and Hsp25 primarily a substrate of the p38-activated kinase MAPK-activated protein kinase 2 [29, 30]. Cytokines significantly increased the kinase activity towards GST-c-jun, whereas there was no effect on Hsp25 phosphorylation (Fig. 2B, C). Surprisingly, cytokines caused a significant reduction in the phosphorylation of GST-Elk-1 (Fig. 2A), suggesting that cytokines suppressed the enzymatic activity of ERK. In MIN6 cells, RX871024 alone caused a modest, but significant increase in GST-c-jun phosphorylation, whereas efaroxan caused suppression in basal GST-c-jun phosphorylation. In combination with cytokines, RX871024, but not efaroxan, increased GST-c-jun phosphorylation by ~35%, as compared to cells exposed to cytokines alone, indicating a potentiating effect of RX871024 on cytokine-stimulated JNK enzymatic activity.

RX871024, but not efaroxan, activates caspases in MIN6 cells, both in the absence and presence of cytokines. Cytokines have been shown to induce caspase-3 activation in pancreatic β-cells [11, 12]. To further investigate the mechanisms underlying the effects of imidazoline compounds on MIN6 cell death, we next evaluated caspase-3 activation. The cells were incubated in the absence or presence of a mixture of cytokines with or without addition of 50 μM RX871024 or efaroxan. After incubation, caspase-3 activity was determined in cell lysates. In accordance with the increase in MIN6 cell death, incubation of cells either with RX871024 alone or with the combination of cytokines for 20 h induced activation of caspase-3 (Fig. 1B). Addition of RX871024 to the mixture of cytokines further increased cytokine-induced caspase-3 activation in MIN6 cells (Fig. 1B). Efaroxan did not affect caspase-3 activation in MIN6

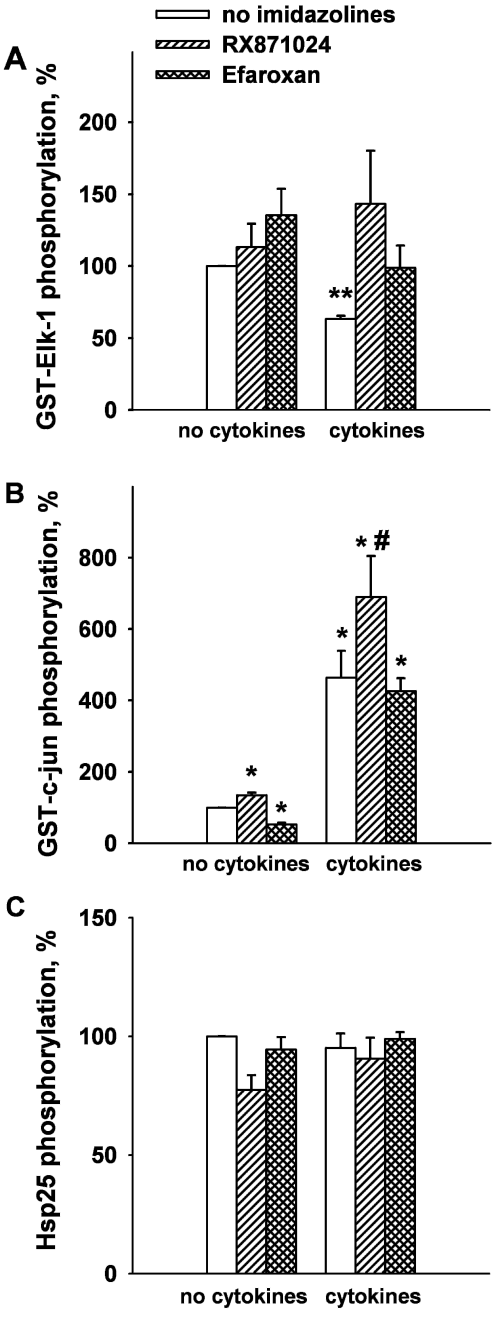

Figure 2. Effect of cytokines and imidazoline compounds on MAPK activity in MIN6 cells. MIN6 cells were incubated in culture medium in the absence or presence of a mixture of cytokines (IL-1β + IFN-γ + TNF-α) with or without 50 μM RX871024 or 50 μM efaroxan for 1 h. Whole-cell lysates were subjected to *in vitro* kinase assay using GST-Elk-1, GST-c-jun or Hsp25 as substrates. Results are means ± SE ($n=3$). Statistical significance between means was assessed by Student's *t*-test for paired values. $^{*}p<0.05$ and $^{**}p<0.005$ vs control; $^{\#}p<0.05$ vs cytokines.

cells either under control conditions or in the presence of a combination of cytokines (Fig. 1B). As the presence of RX871024 induced caspase-3 activation, which was even stronger than that triggered by cytokines (known inducers of apoptosis in insulin-secreting cells), we concluded that the imidazoline induces apoptosis. However, the observed elevation of LDH activity into culture medium indicates the presence of primary or secondary necrosis as well.

The effector caspase-3 is known to be activated through initiator caspases [13, 14]. Therefore, the effects of imidazoline compounds on activation of initiator caspases-1, -8 and -9 in MIN6 cells were evaluated. MIN6 cells were incubated in the absence or presence of the combination of cytokines with or without addition of 50 μM RX871024 or efaroxan. After incubation, caspase-1, -8 and -9 activities were detected in cell lysates. Incubation of MIN6 cells with RX871024 alone caused activation of caspase-1 and to a larger extent activation of caspases-8 and -9 (Fig. 3). This latter finding correlates with induction of caspase-3 activation under the same conditions (Fig. 1B). The combination of cytokines induced activation of caspases-1, -8 and especially -9, after a 20-h incubation (Fig. 3). In line with the increase in MIN6 cell caspase-3 activity, RX871024 further increased cytokine-induced caspase-1, -8 and -9 activations in MIN6 cells after 20-h incubation (Fig. 3). Efaroxan did not affect activation of these caspases in MIN6 cells either in the absence or presence of the combination of cytokines. The data collectively indicate that RX871024 stimulates MIN6 cell death through activation of initiator caspases-1, -8 and -9 and subsequently effector caspase-3.

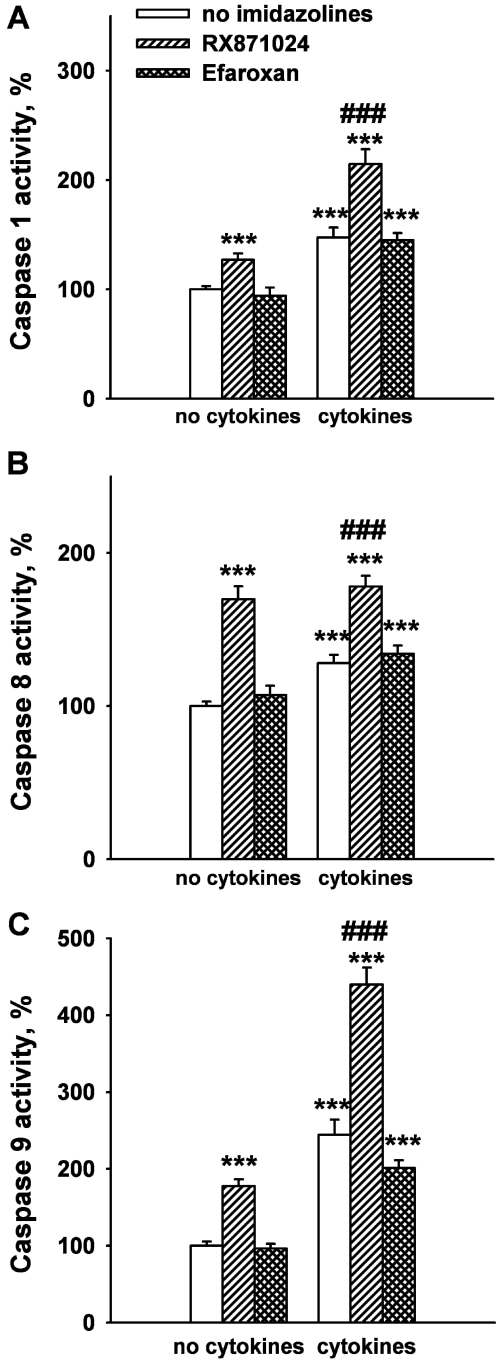

Figure 3. RX871024, but not efaroxan, increases caspase activity in MIN6 cells both in the absence and presence of cytokines. MIN6 cells were incubated in culture medium in the absence or presence of a combination of cytokines (IL-1β + IFN-γ + TNF-α) with or without 50 μM RX871024 or 50 μM efaroxan for 20 h. Caspase-1 (*A*), caspase-8 (*B*), and caspase-9 (*C*) activities were measured by a fluorometric assay. Results are means ± SE (n=3–6). Statistical significance between means was assessed by Student's t-test for unpaired values. $^{***}p<0.0005$ vs control; $^{###}p<0.0005$ vs cytokines.

Discussion

Decreased numbers of β-cells is a feature of both type 1 and type 2 diabetes [1, 15]. On the other hand, β-cells are capable of significant regeneration throughout adult life [31] and the regeneration may actually be induced under certain conditions [22]. However, information on the effects of anti-diabetic drugs on proliferative β-cells is rather restricted. In this study we demonstrated that the insulinotropic imidazoline compound RX871024, but not efaroxan, stimulates death of highly proliferating insulin-secreting cells, exemplified by the MIN6 cell line, in the absence and presence of a combination of pro-inflammatory cytokines. Cytotoxic effect of RX871024 on MIN6 cells was accomplished by JNK activation and activation of initiator caspases-1, -8 and -9 and effector caspase-3. In our investigation we selected 25 mM glucose for culturing of MIN6 cells, since this is the concentration at which this cell line was established [32] and which gives the lowest rate of apoptosis [33, 34]. In this respect this glucose concentration is comparable to

the 11 mM glucose used for culturing of primary β-cells and at which pancreatic β-cell apoptosis is minimal [16].

The results of the present study demonstrate that cytokine-induced MIN6 cell death correlates with caspase-3 activation. Caspase-3 induction has been shown as a downstream event upon induction of β-cell apoptosis [11, 13, 35]. All three tested initiator caspases (caspase-1, -8 and -9) were activated by a mixture of IL-1β, IFN-γ and TNF-α. These pro-inflammatory cytokines induce a network of signal transduction pathways. Upon binding to its receptor, IL-1β activates MAPKs and the transcription factor nuclear factor κB (NF-κB) [36], which in turn induces NO production that contributes to cell death [29, 36]. Although it was previously shown that IL-1β induces ERK activation in primary pancreatic β-cells [29], we were unable to detect any activation of ERK. In contrast, we observed cytokine-induced reduction of ERK activation (Fig. 2A) supposedly caused by the presence of IFN-γ in the cytokine mixture. Indeed, IFN-γ has been shown to reduce the activation of the MAPK ERK in rat pancreatic islets [6]. Moreover, we have previously reported that inhibition of ERK aggravates IL-1β-induced β-cell death [7]. Additionally, IFN-γ induces expression of caspase-1 [37], in line with the observed caspase-1 activation in MIN6 cells by cytokines. There are three known apoptotic signal transduction pathways elicited by the TNF receptor, activation of caspase-8, activation of NF-κB (which can lead to further increase in NO), and activation of the pro-apoptotic MAPK JNK [38]. The caspase-8 activation observed in the present investigation suggests that a signal transduction pathway, starting from the TNF receptor and leading to caspase-8, plays a role in cytokine-stimulated MIN6 cell death. In turn, JNK activation may enable cell death by activating the intrinsic pathway, resulting in the release of cytochrome *c* [39] and thus activation of caspase-9. The present study demonstrates that the combination of IL-1β, IFN-γ and TNF-α induces a strong JNK activation in MIN6 cells, which is associated with caspase-9 activation. Thus, we suggest that JNK-induced activation of the intrinsic (mitochondrial) pathway is an important trigger of MIN6 cell destruction.

In our previous study [10], the protective effect of RX871024 on IL-1β-induced mouse primary β-cell apoptosis was shown to involve inhibition of IL-1β-induced synthesis of NO. However, the results of this investigation do not show any effect of imidazoline compounds RX871024 or efaroxan on NO production induced by the combination of cytokines in the mouse insulin-secreting cell line MIN6. Hence, we conclude that the potentiation of MIN6 cell death by RX871024 in the presence of cytokines does not involve changes in NO production. Indeed, RX871024-triggered reduction of cytokine-induced NO production in primary β-cells was correlated with reduction of cytokine-induced p38 MAPK phosphorylation [21]. We previously demonstrated that p38 is involved in iNOS expression and NO formation [29]. In the present study, we found no effect of cytokines with or without imidazoline compounds on p38 activity in MIN6 cells. In *ob/ob* mouse islets, which contain >90 % β-cells [40], p38 has been shown to be activated with the same combination of cytokines [21]. Therefore, the absence of cytokine-induced p38 activity in MIN6 cells is most likely due to highly proliferative properties of these insulin-secreting cells. Similar observations have been made with mouse βTC3 cells (unpublished results). It is likely that p38 is less sensitive to activation by cytokines in highly proliferating insulin-producing cells than in primary β-cells. Thus, the lack of imidazoline effects on cytokine-induced NO production could be explained by insensitivity of p38 to imidazolines in proliferating insulin-secreting cells.

Our results show that among the two imidazolines RX871024 and efaroxan, only RX871024 potentiates activation of JNK and caspases-1, -3, -8 and -9, both in the absence and presence of a combination of cytokines, which leads to MIN6 cell death. Some imidazoline compounds like idazoxan and phentolamine can activate caspase-3 [26]; however, the signal-transduction pathways of this effector caspase activation by imidazolines were not investigated. We have previously reported that RX871024 induces Ca^{2+} mobilization from the endoplasmic reticulum (ER) in mouse pancreatic β-cells [41]. In contrast, efaroxan does not have such an activity [42]. The effect of RX871024 on ER Ca^{2+} can explain its stimulation of JNK activity in MIN6 cells. Indeed, we have shown [30] that thapsigargin, an agent mobilizing ER Ca^{2+} by inhibition of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA), stimulates JNK activity and potentiates IL-1β-induced JNK activation in INS1-E cells within the same time frame (1 h). As discussed above, stimulation of JNK activity would lead to an increase in caspase-9 activity [39]. This, in turn, would stimulate caspase-3 activation and finally MIN6 cell death.

JNK activation in the presence of RX871024 can also explain elevation in caspase-1 activity as it has been shown that activation of JNK in RINm5F insulin-secreting cells by human amylin causes caspase-1 activation [43]. A stimulatory effect of RX871024 on JNK activity could be responsible for up-regulation of death receptor 5 and subsequent stimulation of caspase-8 activity [44]. Another possible explanation for the observed effect is activation of caspase-8 downstream of caspase-3 [45], following RX871024-

induced activation of caspase-3 by the mechanism discussed above.

Although it was known that β-cells can proliferate [31], this ability was for a long time underestimated. In obese humans the β-cell mass is known to increase, adapting to an elevated metabolic load, by increasing β-cell proliferation and neogenesis from duct cells [15, 46]. A recent investigation by Nir *et al.* [22] implies that under certain conditions β-cells are capable of completely regenerating under diabetic conditions. However, the increase in β-cell proliferation and neogenesis does not outweigh β-cell apoptosis in the course of type 2 diabetes [15]. We and others have previously shown that sulfonylurea compounds used for the treatment for diabetes can actually increase the rate of β-cell apoptosis [16, 47]. Taking this into consideration, it is important that any treatment for diabetes should never compromise the proliferative capacity of β-cells. We previously demonstrated that RX871024 had no effect on either basal or cytokine-induced JNK activation in primary β-cells [21], and did not induce primary β-cell apoptosis [10, 21]. However, the results of the present investigation lead to the conclusion that RX871024 is specifically toxic for proliferating insulin-secreting cells. Moreover, the present data and our previous observations [48] suggest that JNK activation plays an antitumorigenic role in insulinoma cells. Our results on selective destruction of MIN6 insulinoma cells by the imidazoline compound RX871024, without any effect on primary β-cells, suggest that this substance and other compounds showing the same mechanism of action may have a specific antitumorigenic potential against insulinomas. Taken together, our data show that RX871024 induces MIN6 cell death and potentiates cell death induced by the combination of pro-inflammatory cytokines IL-1β, IFN-γ and TNF-α. These effects do not involve changes in p38 activation or cytokine-induced NO production and correlate with potentiation of basal and cytokine-induced JNK activity by RX871024 and subsequent potentiation of caspase-3 activity. Basal and cytokine-induced activation of initiator caspases-1, -8, and -9 were potentiated by RX871024, indicating that both the extrinsic apoptotic pathway and the intrinsic (mitochondrial) apoptotic pathway are activated, leading to MIN6 cell death.

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1 Mandrup-Poulsen, T. (2003) Beta cell death and protection. Ann. N. Y. Acad. Sci. 1005, 32–42.
2 Flodstrom-Tullberg, M., Yadav, D., Hagerkvist, R., Tsai, D., Secrest, P., Stotland, A. and Sarvetnick, N. (2003) Target cell expression of suppressor of cytokine signaling-1 prevents diabetes in the NOD mouse. Diabetes 52, 2696–2700.
3 Chong, M. M., Chen, Y., Darwiche, R., Dudek, N. L., Irawaty, W., Santamaria, P., Allison, J., Kay, T. W. and Thomas, H. E. (2004) Suppressor of cytokine signaling-1 overexpression protects pancreatic beta cells from $CD8^+$ T cell-mediated autoimmune destruction. J. Immunol. 172, 5714–5721.
4 Barral, A. M., Thomas, H. E., Ling, E. M., Darwiche, R., Rodrigo, E., Christen, U., Ejrnaes, M., Wolfe, T., Kay, T. W. and von Herrath, M. G. (2006) SOCS-1 protects from virally-induced CD8 T cell mediated type 1 diabetes. J. Autoimmun. 27, 166–173.
5 Eizirik, D. L. and Mandrup-Poulsen, T. (2001) A choice of death – The signal-transduction of immune-mediated beta-cell apoptosis. Diabetologia 44, 2115–2133.
6 Andersen, N. A., Larsen, C. M. and Mandrup-Poulsen, T. (2000) TNFalpha and IFNgamma potentiate IL-1beta induced mitogen activated protein kinase activity in rat pancreatic islets of Langerhans. Diabetologia 43, 1389–1396.
7 Ammendrup, A., Maillard, A., Nielsen, K., Aabenhus Andersen, N., Serup, P., Dragsbaek Madsen, O., Mandrup-Poulsen, T. and Bonny, C. (2000) The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic beta-cells. Diabetes 49, 1468–1476.
8 Larsen, L., Storling, J., Darville, M., Eizirik, D. L., Bonny, C., Billestrup, N. and Mandrup-Poulsen, T. (2005) Extracellular signal-regulated kinase is essential for interleukin-1-induced and nuclear factor kappaB-mediated gene expression in insulin-producing INS-1E cells. Diabetologia 48, 2582–2590.
9 Hadjivassiliou, V., Green, M. H., James, R. F., Swift, S. M., Clayton, H. A. and Green, I. C. (1998) Insulin secretion, DNA damage, and apoptosis in human and rat islets of Langerhans following exposure to nitric oxide, peroxynitrite, and cytokines. Nitric Oxide 2, 429–441.
10 Zaitsev, S. V., Appelskog, I. B., Kapelioukh, I. L., Yang, S. N., Kohler, M., Efendic, S. and Berggren, P. O. (2001) Imidazoline compounds protect against interleukin 1beta-induced beta-cell apoptosis. Diabetes 50 Suppl 1, S70–76.
11 Kohler, M., Zaitsev, S. V., Zaitseva, I. I., Leibiger, B., Leibiger, I. B., Turunen, M., Kapelioukh, I. L., Bakkman, L., Appelskog, I. B., de Monvel, J. B., Imreh, G. and Berggren, P. O. (2003) On-line monitoring of apoptosis in insulin-secreting cells. Diabetes 52, 2943–2950.
12 Augstein, P., Bahr, J., Wachlin, G., Heinke, P., Berg, S., Salzsieder, E. and Harrison, L. C. (2004) Cytokines activate caspase-3 in insulinoma cells of diabetes-prone NOD mice directly and *via* upregulation of Fas. J. Autoimmun. 23, 301–309.
13 Chandra, J., Zhivotovsky, B., Zaitsev, S., Juntti-Berggren, L., Berggren, P. O. and Orrenius, S. (2001) Role of apoptosis in pancreatic beta-cell death in diabetes. Diabetes 50 Suppl. 1, S44–47.
14 Riedl, S. J. and Shi, Y. (2004) Molecular mechanisms of caspase regulation during apoptosis. Nat. Rev. Mol. Cell. Biol. 5, 897–907.
15 Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A. and Butler, P. C. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52, 102–110.
16 Efanova, I. B., Zaitsev, S. V., Zhivotovsky, B., Kohler, M., Efendic, S., Orrenius, S. and Berggren, P. O. (1998) Glucose and tolbutamide induce apoptosis in pancreatic beta-cells: A process dependent on intracellular Ca^{2+} concentration. J. Biol. Chem. 273, 33501–33507.
17 Zaitsev, S. V., Efanov, A. M., Efanova, I. B., Larsson, O., Ostenson, C. G., Gold, G., Berggren, P. O. and Efendic, S. (1996) Imidazoline compounds stimulate insulin release by

inhibition of K(ATP) channels and interaction with the exocytotic machinery. Diabetes 45, 1610–1618.
18 Zaitsev, S. V., Efanov, A. M., Raap, A., Efanova, I. B., Schloos, J., Steckel-Hamann, B., Larsson, O., Ostenson, C. G., Berggren, P. O., Mest, H. J. and Efendic, S. (1999) Different modes of action of the imidazoline compound RX871024 in pancreatic beta-cells. Blocking of K^+ channels, mobilization of Ca^{2+} from endoplasmic reticulum, and interaction with exocytotic machinery. Ann. N. Y. Acad. Sci. 881, 241–252.
19 Efendic, S., Efanov, A. M., Berggren, P. O. and Zaitsev, S. V. (2002) Two generations of insulinotropic imidazoline compounds. Diabetes 51 Suppl. 3, S448–454.
20 Rustenbeck, I., Kopp, M., Ratzka, P., Leupolt, L. and Hasselblatt, A. (1999) Imidazolines and the pancreatic B-cell. Actions and binding sites. Ann. N. Y. Acad. Sci. 881, 229–240.
21 Zaitseva, I. I., Sharoyko, V., Storling, J., Efendic, S., Guerin, C., Mandrup-Poulsen, T., Nicotera, P., Berggren, P. O. and Zaitsev, S. V. (2006) RX871024 reduces NO production but does not protect against pancreatic beta-cell death induced by proinflammatory cytokines. Biochem. Biophys. Res. Commun. 347, 1121–1128.
22 Nir, T., Melton, D. A. and Dor, Y. (2007) Recovery from diabetes in mice by beta cell regeneration. J. Clin. Invest. 117, 2553–2561.
23 Ackermann, A. M. and Gannon, M. (2007) Molecular regulation of pancreatic beta-cell mass development, maintenance, and expansion. J. Mol. Endocrinol. 38, 193–206.
24 Ablamunits, V., Sherry, N. A., Kushner, J. A. and Herold, K. C. (2007) Autoimmunity and beta cell regeneration in mouse and human type 1 diabetes: The peace is not enough. Ann. N. Y. Acad. Sci. 1103, 19–32.
25 Sharoyko, V. V., Zaitseva, I. I., Varsanyi, M., Portwood, N., Leibiger, B., Leibiger, I., Berggren, P. O., Edendic, S. and Zaitsev, S. V. (2005) Monomeric G-protein, Rhes, is not an imidazoline-regulated protein in pancreatic beta-cells. Biochem. Biophys. Res. Commun. 338, 1455–1459.
26 Rustenbeck, I., Krautheim, A., Jorns, A. and Steinfelder, H. J. (2004) Beta-cell toxicity of ATP-sensitive K^+ channel-blocking insulin secretagogues. Biochem. Pharmacol. 67, 1733–1741.
27 Mourtada, M., Elliott, J., Smith, S. A. and Morgan, N. G. (2000) Effects of imidazoline binding site ligands on the growth and viability of clonal pancreatic beta-cells. Naunyn Schmiedebergs Arch. Pharmacol. 361, 146–154.
28 Chan, S. L. and Morgan, N. G. (1990) Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. Eur. J. Pharmacol. 176, 97–101.
29 Larsen, C. M., Wadt, K. A., Juhl, L. F., Andersen, H. U., Karlsen, A. E., Su, M. S., Seedorf, K., Shapiro, L., Dinarello, C. A. and Mandrup-Poulsen, T. (1998) Interleukin-1beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. J. Biol. Chem. 273, 15294–15300.
30 Storling, J., Zaitsev, S. V., Kapelioukh, I. L., Karlsen, A. E., Billestrup, N., Berggren, P. O. and Mandrup-Poulsen, T. (2005) Calcium has a permissive role in interleukin-1beta-induced c-jun N-terminal kinase activation in insulin-secreting cells. Endocrinology 146, 3026–3036.
31 Finegood, D. T., Scaglia, L. and Bonner-Weir, S. (1995) Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. Diabetes 44, 249–256.
32 Miyazaki, J., Araki, K., Yamato, E., Ikegami, H., Asano, T., Shibasaki, Y., Oka, Y. and Yamamura, K. (1990) Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 127, 126–132.
33 Muller, D., Jones, P. M. and Persaud, S. J. (2006) Autocrine anti-apoptotic and proliferative effects of insulin in pancreatic beta-cells. FEBS Lett. 580, 6977–6980.
34 Papadimitriou, A., King, A. J., Jones, P. M. and Persaud, S. J. (2007) Anti-apoptotic effects of arachidonic acid and prostaglandin E2 in pancreatic beta-cells. Cell Physiol. Biochem. 20, 607–616.
35 Todaro, M., Di Gaudio, F., Lavitrano, M., Stassi, G. and Papaccio, G. (2003) Islet beta-cell apoptosis triggered *in vivo* by interleukin-1beta is not related to the inducible nitric oxide synthase pathway: Evidence for mitochondrial function impairment and lipoperoxidation. Endocrinology 144, 4264–4271.
36 Mandrup-Poulsen, T. (2003) Apoptotic signal transduction pathways in diabetes. Biochem. Pharmacol. 66, 1433–1440.
37 Karlsen, A. E., Pavlovic, D., Nielsen, K., Jensen, J., Andersen, H. U., Pociot, F., Mandrup-Poulsen, T., Eizirik, D. L. and Nerup, J. (2000) Interferon-gamma induces interleukin-1 converting enzyme expression in pancreatic islets by an interferon regulatory factor-1-dependent mechanism. J. Clin. Endocrinol. Metab. 85, 830–836.
38 Jin, Z. and El-Deiry, W. S. (2005) Overview of cell death signaling pathways. Cancer Biol. Ther. 4, 139–163.
39 Herr, I. and Debatin, K. M. (2001) Cellular stress response and apoptosis in cancer therapy. Blood 98, 2603–2614.
40 Nilsson, T., Arkhammar, P., Hallberg, A., Hellman, B. and Berggren, P. O. (1987) Characterization of the inositol 1,4,5-trisphosphate-induced Ca^{2+} release in pancreatic beta-cells. Biochem. J. 248, 329–336.
41 Efanova, I. B., Zaitsev, S. V., Brown, G., Berggren, P. O. and Efendic, S. (1998) RX871024 induces Ca^{2+} mobilization from thapsigargin-sensitive stores in mouse pancreatic beta-cells. Diabetes 47, 211–218.
42 Squires, P. E., Hills, C. E., Rogers, G. J., Garland, P., Farley, S. R. and Morgan, N. G. (2004) The putative imidazoline receptor agonist, harmane, promotes intracellular calcium mobilisation in pancreatic beta-cells. Eur. J. Pharmacol. 501, 31–39.
43 Zhang, S., Liu, J., Dragunow, M. and Cooper, G. J. (2003) Fibrillogenic amylin evokes islet beta-cell apoptosis through linked activation of a caspase cascade and JNK1. J. Biol. Chem. 278, 52810–52819.
44 Higuchi, H., Grambihler, A., Canbay, A., Bronk, S. F. and Gores, G. J. (2004) Bile acids up-regulate death receptor 5/TRAIL-receptor 2 expression *via* a c-Jun N-terminal kinase-dependent pathway involving Sp1. J. Biol. Chem. 279, 51–60.
45 Hui, H., Dotta, F., Di Mario, U. and Perfetti, R. (2004) Role of caspases in the regulation of apoptotic pancreatic islet beta-cells death. J. Cell Physiol. 200, 177–200.
46 Rhodes, C. J. (2005) Type 2 diabetes-a matter of beta-cell life and death? Science 307, 380–384.
47 Maedler, K., Carr, R. D., Bosco, D., Zuellig, R. A., Berney, T. and Donath, M. Y. (2005) Sulfonylurea induced beta-cell apoptosis in cultured human islets. J. Clin. Endocrinol. Metab. 90, 501–506.
48 Storling, J., Allaman-Pillet, N., Karlsen, A. E., Billestrup, N., Bonny, C. and Mandrup-Poulsen, T. (2005) Antitumorigenic effect of proteasome inhibitors on insulinoma cells. Endocrinology 146, 1718–1726.