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Non-receptor tyrosine kinase inhibitors enhances β-cell survival by suppressing the PKCδ signal transduction pathway in streptozotocin-induced β-cell apoptosis

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ABSTRACT

GNF-2 and GNF-5 are members of a new class of non-receptor tyrosine kinases inhibitors that possess excellent selectivity towards imatinib-resistant mutations found in chronic myeloid leukemia patients. On the other hand recent reports implicate abnormal tyrosine kinase signaling in β-cell death in Type I and Type II diabetes. In this work we determined the effects of GNF-2, GNF-5 on pancreatic β-cell death caused by streptozotocin (STZ). STZ treatment causes apoptosis of INS-1 cells by activation of intracellular ROS, c-jun N-terminal kinase (JNK), caspase 3, and caspase 3-dependent activation of protein kinase C delta (PKCδ). GNF-2 and GNF-5 increased cell viability and attenuated STZ-induced intracellular ROS and significantly reduced the activation of JNK, caspase 3, and caspase 3-dependent activation of PKCδ. In studies with intact mice, GFN-2 and GNF-5 prevented the loss of beta cells and the increase in blood glucose produced by STZ-treated control mice. Furthermore, immunohistochemical analysis revealed that GNF-2 and GNF-5 increased insulin protein levels in STZ-treated mice when compared with control mice. These findings suggest that non-receptor tyrosine kinase inhibitors provide a new approach for the treatment of new-onset Type I and Type II diabetes.

1. Introduction

Type 1 diabetes (T1D) is an auto-inflammatory disease in which pancreatic β-cell dysfunction and damage is mediated by cytotoxic pro-inflammatory cytokines [1]. In comparison, Type 2 diabetes (T2D) is a more complex metabolic disorder characterized by peripheral insulin resistance, hyperglycemia, obesity and cytokines, leading to a relative lack of insulin and β-cell failure [2]. Apoptosis is the end-point of β-cell death in both forms of the disease. STZ administration in vivo causes impaired glucose homeostasis as a consequence of hypoinsulinaemia due to deterioration of β-cell mass and abnormalities in β-cell function [3]. At the cellular level, STZ has been shown to impair glucose oxidation and decrease insulin biosynthesis and secretion [4]. As a result of STZ exposure, β-cells will eventually experience loss of glucose responsiveness, followed by permanent cell damage and death. The β-cell specificity of STZ action can be attributed to its mechanism of uptake. STZ enters the cytoplasm via the plasma membrane-bound glucose transporter Type 2 (GLUT2). GLUT2 is the major glucose transporter expressed in β-cells and therefore facilitates their vulnerability to STZ [5,6]. The cytotoxic action of STZ is primarily attributed to the alkylation of DNA and its resulting fragmentation. However, evidence suggests that the effects are also strongly mediated by the accumulation of superoxide, hydroxyl radicals, and nitric oxide. Pancreatic beta-cells intrinsically express relatively low levels of protective antioxidant enzymes in comparison to other tissues [7,8], another factor that contributes to their sensitivity to STZ. In addition, pro-inflammatory signaling pathways are known to involve activation of protein kinase C (PKC) in that PKCδ particularly

Abbreviations: c-Abl, cellular Abelson tyrosine kinase; STZ, streptozotocin; MMP, mitochondrial membrane potential; CML, chronic myeloid leukemia; JNK, c-Jun N-terminal kinase; PKCδ, protein kinase c delta; ROS, reactive oxygen species; GNF-2, GNF-2 3-[6-[[4-(Trifluoromethoxy) Phenyl] amino]-4-pyrimidinyl] benzamide; GNF-5, GNF-5 N-(2-Hydroxyethyl)-3-[6-[[4-(Trifluoromethoxy) Phenyl]amino]-4-pyrimidinyl] benzamide.

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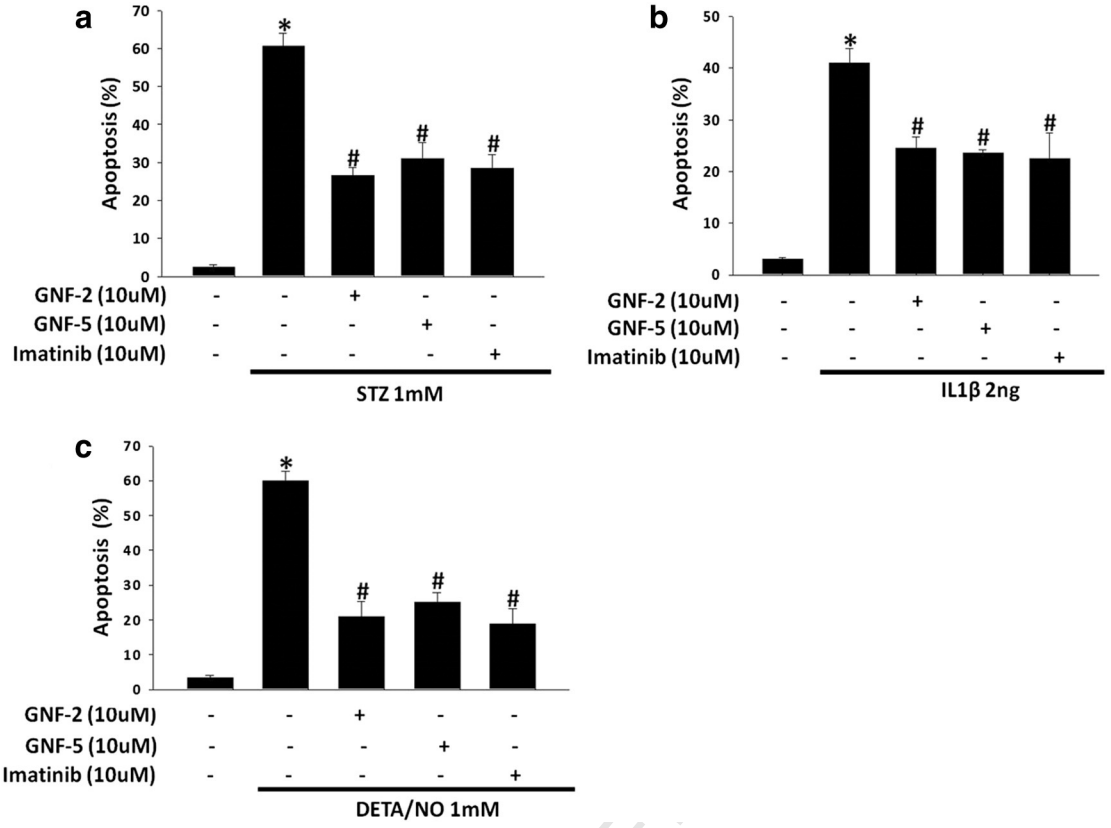

Fig. 1. GNF-2, GNF-5 and imatinib protect β-cells against streptozotocin induced apoptosis. INS-1 cells were treated with indicated concentrations of GNF-2, GNF-5 and imatinib for 90 min and then exposed to streptozotocin (STZ) 1 mM, IL1β 2 ng and DETA/NO 1 mM for 9 (a) and 24 h (b, c) respectively. Apoptosis was measured by TUNEL in situ cell death detection kit using fluorescence microscopy. Experiments were performed in triplicate. Values represent means ± SEM ($^{*}P < 0.0001$ vs. control, $^{\#}P < 0.005$ vs. STZ).

plays a direct catalytic role in mediating the biochemical aspects of apoptosis. The underlying mechanism of activation involves proteolytic cleavage of PKCδ by caspase 3 [9,10]. Both interleukin-1β (IL-1β) and STZ-induced caspase dependent cleavage of PKCδ are important in the distal β-cell apoptotic process [11]. Furthermore, phosphorylation of PKCδ at Tyr311 by c-Abl is important for hydrogen peroxide-induced apoptosis [12,13]. However, the intracellular signaling pathway upstream and downstream of PKCδ activation and the biochemical mechanism by which nuclear damage by STZ culminates in cell death have not been clearly elucidated. From these findings it is apparent that c-Abl can sense and integrate information from multiple signaling pathways in different cellular compartments and then interact with downstream effector proteins to induce cell cycle arrest and apoptosis. As a result of the insight provided by these findings, we hypothesized that c-Abl inhibitors may protect β-cells against the oxidative stress that causes diabetes. The aim of the present investigation was to determine the effect of new non-ATP competitive c-Abl kinase inhibitors GNF-2 and GNF-5 against STZ-induced β-cell death. In addition we used imatinib an ATP competitive c-Abl kinase inhibitor as a positive control.

2. Materials and methods

2.1. Chemicals

GNF2, GNF5 and imatinib were obtained from Professor Taebo Sim (Korea Institute of Science and Technology, South Korea). DiOC6 and SP600125 (JNK inhibitor) were purchased from Sigma-Aldrich (St Louis, MO, USA). Z-VAD-FMK was obtained from Tocris Bioscience (Ellisvile, MS, USA).

2.2. Cell culture

The INS-1 rat insulinoma cell line (INS-1) was cultured in 5% CO_2 at 37 °C in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 units/ml penicillin, and 100 μg/ml streptomycin.

2.3. Cell viability assay

Apoptosis were assessed by TUNEL in-situ cell death detection kit (Roche, Basel, Switzerland). Briefly, INS-1 cells were grown on glass cover slips in 6-well plate and preincubated with indicated concentrations of GNF-2, GNF-5 and imatinib for 90 min and then exposed to STZ (1 mM), IL1β (2 ng) and DETA/NO (1 mM) for the indicated time points. After incubation cells were washed with 1X PBS for 3 times and fixed with 2% paraformaldehyde for 15 min and then permeabilized with 0.2% Triton X-100 for 10 min at room temperature. After permeabilization, cells were washed again with PBS and processed further according to manufactures instructions and image was captured using fluorescence microscopy.

2.4. Assessment of cellular ROS & mitochondrial membrane potential

Cellular reactive oxygen species was measured using 2, 7-dichlorodihydrofluorescein diacetate (DCF-DA, Molecular Probes, Invitrogen, USA). After incubated with experimental condition, cells were washed with PBS and then incubated in the dark for 15 min

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