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Salvianolic acid B improves insulin secretion from interleukin 1β-treated rat pancreatic islets: The role of PI3K-Akt signaling

Safoura Raoufi[1,2*], Tourandokht Baluchnejadmojarad[2]

1. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

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ABSTRACT

Background and Objective: Oxidative stress induced by proinflammatory cytokines such as IL-1β plays a major role in β-cell destruction in diabetes type 1. Salvianolic acid B (Sal B) is a polyphenolic compound with antioxidant and protective effects. Thus, objective of this study was to assess the protection exerted by Sal B on isolated rat islets exposed to IL-1β and to investigate an underlying mechanism in vitro.

Materials and Methods: Isolation of pancreatic islets was done by using the collagenase digestion method. Isolated rat islets were divided into 6 groups including: 1. control, 2. interleukin-1β treated, 3 and 4. interleukin-1β treated+ Sal B, 5 and 6. interleukin-1β treated+ Sal B+ PKB and PI3K inhibitors. Interleukin-1β (1 U/ml) was used to induce cytotoxicity after pretreatment with two doses of Sal B (50 μM and 100 μM) and application of each inhibitors was before Sal B.

Results: IL-1β significantly decreased insulin secretion from isolated islets. Pretreatment with Sal B ameliorated the effect of IL-1β on glucose stimulated insulin secretion in a concentration dependent manner. Inhibitors of PKB and PI3K both abolished these improving effect of Sal B.

Conclusion: Sal B that has antioxidant, anti inflammatory and anti apoptotic properties, provided resistance to pancreatic β-cell dysfunction from cytokine in part via PI3K/Akt pathway. The findings represent that it is a promising agent for prevention of β-cell dysfunction in type 1 diabetes.

1. Introduction

Type 1 diabetes is an autoimmune disorder that brings about insulin secreting β-cells destruction (1). There are numerous studies showing that infiltrated-immune cells in and around pancreatic islets play a prominent role in its pathogenesis(2). Pro-inflammatory cytokines involved in diabetes progression are interleukin-1β (IL-1β), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) that released by these immune cells (3). It has been mentioned that free radical production raised by these inflammatory cytokines take part in destruction of pancreatic β-cells (4). Pancreatic islet β-cells are highly vulnerable to injury by free radicals, because of low antioxidant enzyme levels (5), so islets may be damaged by apoptosis due to imbalance between oxidant/antioxidant systems (6). Cytotoxic effect of cytokines on β-cells is demonstrated to induce by reactive oxygen species (ROS) generation and lipid peroxidation in the human islets (7). ROS enhancement in pancreatic β-cells impairs the islets and decreases gene expression and secretion of insulin (7).

***Corresponding Author:** *Safoura Raoufi*
Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
Email: safooraraufi@yahoo.com

Salvianolic acid B (Sal B) is a polyphenolic substance isolated from the medicinal plant *Salvia miltiorrhiza* that has strong antioxidant effect because of its ability to scavenging free radicals (8-10). In addition to protecting pancreatic β-cells against cytotoxicity(11) Sal B can prevent the inflammatory damage (12) and apoptosis (13) in several organs. We recently reported that Sal B can act in vivo as an antidiabetic agent by increasing the GSH (glutathione) level and CAT (catalase) activity, lowering MDA (malondialdehyde) and restricting apoptosis activity in rat pancreas (14). Protein kinase B (PKB/Akt) that is activated by phosphatidylinositol 3-kinase (PI3K) induces phosphorylation and has important role in cell survival, is expressed in pancreatic β-cells (15). Several evidences have implicated that activation of PI3K/Akt pathway leads to β-cells protection against toxicity (15-19). Some studies have revealed that the protective effects of Sal B are in part mediated through the PI3K/Akt signaling pathway (20-22). Therefore, the goal of the present study was to investigate the protective effect of Sal B against IL-1β induced dysfunction in isolated pancreas islets with deliberation of PI3K/Akt signaling pathway.

2. Materials and Methods

2.1. Animals

Male Wistar rats (250–300 g) obtained from Pasteur Institute of Iran were kept under 12 hour light-dark cycle with free access to water and food. The experimental protocols were confirmed by the Iran University of Medical Sciences ethics committee.

2.2. Islet isolation, culture and treatment

The islet isolation was performed by the collagenase technique as described elsewhere (23) with slight modification.

Rats were anesthetized with intraperitoneal (ip) injection of ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg) and the abdomen was opened. The common bile duct was cannulated with a polyethylene catheter and the entrance of duct to duodenum was clamped. Afterwards, 10 ml cold Hanks' balanced salt solution (HBSS) [136 mM NaCl, 5.36 mM KCl, 1.26 mM CaCl2, 0.8 mM MgSO4, 0.33 mM Na2HPO, 0.44 mM KH_2PO_4 , 4.16 mM $NaHCO_3$ (Merck, Germany), pH = 7.4] containing 0.5 mg/ml of collagenase P (Roche, Germany) was injected into the common bile duct (24).

The distended pancreas was removed, placed into a falcon tube and incubated in a water bath at 37°C for 17 min. Digestion was stopped by addition of cold HBSS, then the tube was shaken well for 1 min. After three rinses with cold HBSS, islets were collected via hand picking (under a BlueLight stereomicroscope, USA). RPMI 1640 medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and 8.3 mmol/l glucose was added to the obtained islets. Then, they were kept at 37˚C with 0.5% CO2 for 3 hours before use (25, 26). In the next stage, groups of 10 islets were incubated for 24 h in medium containing IL-1β (1 U/mL) in the presence or absence of Sal B (Sigma-Aldrich, St. Louis, MO).

Six groups of 10 islets were evaluated for each experimental group, 1. Control (non-treated), 2. Toxic (IL-1β treated), 3 and 4. toxic+Sal B (different doses of Sal B), 5. toxic+Sal B+AKT inhibitor (MK-2206), 6. toxic+Sal B+PI3K inhibitor (LY294002). Pretreatment with two different doses of Sal B (50 μM and 100 μM) was done 1 hour before addition of IL-1β and inhibitors were used 1 hour before Sal B.

2.3. Glucose-stimulated insulin secretion (GSIS) assay

Glucose-stimulated insulin secretion assay was carried out at two different concentrations of glucose [5.6 mM (basal concentration) and 16.7 mM (stimulant concentration)]. Groups of ten islets for each glucose concentrations were incubated in Krebs Ringer solution [111 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 24 mM NaHCO3 (Merck, Germany), 10 mM HEPES, 0.5 g/dl BSA (Sigma, USA), pH 7.4] (24) containing 5.6 and 16.7 mM of glucose at 37˚C for 60 minutes. At the end, the supernatant was collected and reserved at -70°C for insulin assay using ELISA technique (EIA Kit, Cayman Chemical).

2.4. Statistical analysis

The differences between groups were determined using one-way analysis of variance (ANOVA) followed by post-hoc test. The results

were expressed as mean ± standard error of mean (SEM). P value below 0.05 was considered to be significant.

3. Results

Sal B dose-dependently prevented cytokine-elicited impairment in glucose-stimulated insulin secretion from rat islets. Figure 1 showed that insulin output from all groups of isolated islets was significantly more in the presence of 16.7 mM glucose concentration (stimulant dose) than 5.6 mM of glucose concentration (basal dose). We established IL-1β mediated islet dysfunction. Insulin secretion was markedly impaired in islets previously exposed to cytokines. Insulin release from IL-1β treated islets was significantly decreased at both glucose concentrations as compared to control islets. When the islets were pretreated with Sal B for 1 h before the addition of IL-1β, glucose-stimulated insulin secretion was ameliorated.

As shown in Figure 1, treatment of IL-1β exposed islets with lower dose of Sal B (50 μM) increased insulin release with 16.7 mM glucose, whereas for higher dose of Sal B (100 μM), this effect was seen at both glucose concentrations (5.6 mM and 16.7 mM).

Insulin secretion in islets exposed to IL-1β was restored to normal values by Sal B pretreatment at a higher dose (no significant difference with control) but not at lower (significant decrease as compared with control).

To examine the probable role of PI3K/Akt pathway in the effect of Sal B on insulin release, PKB inhibitor (PKBI) and PI3K inhibitor (PI3KI) were administrated to Sal B treated islets that were exposed to IL-1β separately.

PKB I administration significantly reduced insulin secretion from these islets. Insulin release in these islets had no significant deference with untreated IL-1β exposed islets. Similar result was also observed with PI3KI.

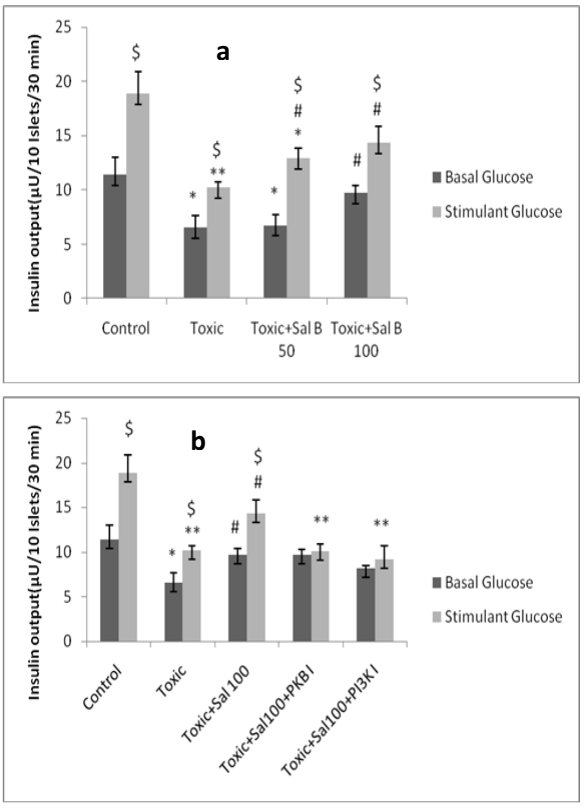

Figure 1. The insulin output from pancreatic isolated islets at different glucose concentrations.

a. Effect of Sal B on insulin secretion in islets exposed to IL-1β.

b. Effect of PKB I and PI3K I on insulin secretion in Sal B treated islets that were exposed to IL-1β.

Values are represented as mean ± SEM.

$ $p<0.05$, significant difference versus basal glucose concentration of the same group.

* $p<0.05$, ** $p<0.01$, significant difference versus control group in the same glucose concentration.

# $p<0.05$, significant difference versus toxic group in the same glucose concentration.

4. Discussion

Most current evidence points that inflammatory cytokines released by immune cells infiltrating pancreatic islets during an autoimmune reaction are the main mediators in the development of type 1 diabetes (27-29). IL-1β is one of these cytokines that is involved in β -cell destruction (30). In the current study, we evaluated IL-1β induced impairment in insulin secretion in pancreatic islets. We assessed glucose-stimulated insulin secretion at basal and stimulant concentrations of glucose.

Insulin secretion with high glucose concentration, was markedly more than with basal concentrations of glucose. These results confirm previous studies that insulin secretion level is dependent to glucose concentration (31, 32). It is evidenced that insulin secretion from pancreatic islets is regulated by glucose uptake by β-cells that cause ATP to produce. Closure of ATP-sensitive K+ channels leads to cell depolarization, voltage-dependent Ca2+ channels activation and finally insulin exocytosis (33, 34).

In our study, IL-1β induced toxicity lowered insulin secretion at both glucose concentration. Similar to our findings, Novelli reported that at 16.7 mM of glucose, insulin secretion increases about threefold over basal, but was markedly impaired in islets previously exposed to cytokines (35). Just as deterioration of in vitro glucose-stimulated insulin manufacturing by IL-1β that has been documented (36).

Oxygen free radical production, lipid peroxidation, and the generation of aldehydes that are toxic to the β-cells have been suggested to be involved in cytokine-induced islet β-cell destruction (37). Researchers have demonstrated production of free radicals by pancreatic β-cells in reaction to pro-inflammatory cytokines (4). It has been pointed out that extreme ROS brings about β-cell dysfunction and insulin synthesis and secretion deficiency (38). The antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) in β cells are partly poor, thus they fail to prevent the oxidative stress sufficiently (39, 40). Therefore, edifying antioxidant protection mechanisms in pancreatic islets helps them to overcome oxidative stress. Several antioxidant compounds have been reported to significantly inhibit the islet cytotoxic effects of inflammatory cytokines (41). Similar to this, here we found that prior addition of Sal B to the islets provided protection against IL-1β cytotoxic effects dose dependently as this effect for higher dose of Sal B was more intense. Growing studies have revealed activation of PI3K/Akt pathway as an effective agent for protecting β-cells against toxicity (15, 16, 18, 19) and several preserving effects of *Sal B* have been mediated by this pathway. So, we inspected whether the PI3K/Akt pathway is involved in the protective effect of Sal B against IL-1β induced dysfunction in β cells.

Islets' pretreatment with PI3K and Akt inhibitors both impeded Sal B-induced insulin secretion amelioration under IL-1β toxicity condition. Comparable to these results PI3K/Akt signaling pathway role in Sal B protective effects against toxic agents have been reported. Sal B has inhibited H2O2 induced apoptosis by regulating PI3K/Akt signaling in endothelial cell (21). Protective effect of Sal B against arsenic trioxide-induced damage in cardiac cells by means of this pathway is reported too (22). Suppression of oxidative stress, inflammation and apoptosis has played a role in Sal B protecting effects induced by PI3K/Akt signaling pathway (20, 21).

Our previous study showed that Sal B administration to diabetic rats enhanced serum insulin through reinforced anti-oxidative defense system, weakened oxidative stress and maintained pancreatic islets by apoptosis inhibition (14). Potent antioxidant properties of Sal B such as free radicals scavenging and lipid peroxidation decreasing have been shown (42). Chen reported that Sal B enhances SOD activity and reduces MDA production, even stronger than Vit C (43). Furthermore, this fact that Sal B rises antioxidant defensive system against various free radicals (44) can verify protective effect of this compound in our study.

Gao reported that Sal B attenuates the inflammation induced by some toxic agents via reducing the expression of TGF-β_1 that is an inflammatory mediator (45). Moreover, Sal B could suppress the pro-inflammatory cytokines IL-1β and TNF-a expression (46). Therefore, anti-inflammatory characteristics of Sal B may have a role in its advantageous effect in this study.

Balancing the expression of Bcl-2 family members, down-regulating Bax expression, reducing the release of cytochrome C, decreasing the expression of p53 and inhibiting activated caspase-3 are another effects of Sal B are responsible for anti-apoptotic effect of Sal B (13, 43, 47). So, the same anti-apoptotic mechanisms may have occurred in our study by Sal B to protect against IL-1β.

In conclusion, this study indicated that Salvianolic acid B improves islet function against IL-1β, the main proinflammatory cytokine in type 1 diabetes progression. PI3K/Akt signaling pathway besides antioxidant, anti inflammatory, and anti-apoptotic properties of Sal B may be involved in this result.

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