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Dose-related cytoprotective effect of α-lipoic acid on hydrogen peroxide-induced oxidative stress to pancreatic beta cells

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Abstract

α-Lipoic acid (α-LA), an antioxidant used for diabetic polyneuropathy, was reported to induce AMP-activated protein kinase activation and reductions in insulin secretion in pancreatic beta-cells at high concentrations ($\geq$ 500 μmol/l). This study investigated whether α-LA has a protective role under oxidative stress in beta-cells and its effect is dose-related. In INS-1 cells treated with α-LA (150-1200 μmol/l) for 24 h, α-LA itself ($\geq$ 300 μmol/l) induced apoptotic death dose-dependently. However, pre-treatment with 150 and 300 μmol/l α-LA reduced the hydrogen peroxide-induced apoptosis in INS-1 cells and isolated islets. α-LA alleviated hydrogen peroxide-induced reactive oxygen species production, mitochondrial membrane depolarization and c-JNK activation in beta-cells. α-LA induced phosphoinositide 3-kinase-dependent Akt phosphorylation in INS-1 cells. While α-LA is harmful to beta-cells at high concentrations *in vitro*, it has potential cytoprotective effects on beta-cells under oxidative stress as in diabetes by its antioxidant properties and possibly by Akt phosphorylation at clinically relevant concentrations.

Keywords: *Akt phosphorylation, antioxidant, apoptosis, hydrogen peroxide, α-lipoic acid, pancreatic beta-cell*

Abbreviations: *α-LA, α-lipoic acid; AMPK, AMP-activated protein kinase; AO, acridine orange; AV, annexin V-FITC; DHLA, dihydrolipoic acid; GSI, glucose stimulation index; H_2DCFDA, 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate; MMP, $\Delta\psi m$, Mitochondrial membrane potential; PI, propidium iodide; ROS, reactive oxygen species.*

Introduction

α-Lipoic acid (α-LA, 1,2-dithiolane-3-pentanoic acid), also known as thioctic acid, is a naturally occurring short chain fatty acid with sulphydryl groups. α-LA is a powerful compound with diverse biological and pharmacological properties. α-LA, along with its major metabolite dihydrolipoic acid (DHLA), is a potent antioxidant with many beneficial characteristics, including free-radical scavenging, metal-chelating activity, interaction with other antioxidants and suppressive effects on redox-sensitive gene expression [1–3]. The antioxidant effect of α-LA is utilized in diseases associated with increased oxidative stress, including diabetic neuropathy [4].

The beneficial properties of α-LA in the treatment of diabetes include not only the relief of symptoms of diabetic polyneuropathy but also the partial amelioration of insulin resistance by increasing insulin-stimulated glucose uptake [5,6]. Recently, the anti-obesity

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and anti-hyperglycemic properties of α-LA at relatively high doses were linked to AMP-activated protein kinase (AMPK) in a wide variety of tissues, including hypothalamus and skeletal muscles [7–9]. In beta-cells, it has been shown that α-LA induces AMPK activation resulting in reductions in insulin secretion, insulin content and beta-cell growth [10]. The prolonged AMPK activation in beta cells was reported to induce enhanced production of mitochondria-derived oxygen radicals and onset of the intrinsic mitochondrial apoptosis pathway [11]. The concentration of α-LA for significant induction of AMPK activation in islet cells was at least 500 μmol/l [10], which is far above the plasma concentration reached by α-LA doses used for diabetic neuropathy [12–15].

Literature data indicate that α-LA effect depends on its dose [1]. For example, in tumour cells, α-LA at low concentrations (1 μmol/l) increased cell proliferation rate, while α-LA at high concentrations (100 μmol/l) exhibited a distinct anti-proliferative effect [16]. In addition, α-LA could act as a pro-oxidant at particular concentrations in chemical systems and living tissues [17,18], such as increasing reactive oxygen species (ROS) in isolated rat soleus muscle [19] and increasing ROS-induced mitochondrial permeability transition in rat liver mitochondria [20]. Although α-LA itself can be detrimental in the absence of other pro-oxidants, this compound has been shown to exhibit beneficial protective properties under conditions of oxidative stress, such as diabetes. In diabetic rats, α-LA improved albuminuria and pathologic changes in kidneys by reducing oxidative stress, while in healthy rats α-LA acted as a pro-oxidant contributing to renal dysfunction [21].

The decline in beta-cell function and mass is known to contribute to the progressive nature of type 2 diabetes. The beta-cell loss in type 2 diabetes is accompanied by a marked increase in beta-cell apoptosis, as shown in human pancreas autopsy specimens and in isolated islets [22,23]. Chronic oxidative stress is considered as one of the central mechanisms for glucose and lipid toxicity in pancreatic beta-cells in diabetes [24,25]. Considering the clinical use of α-LA for the treatment of diabetic neuropathy and the importance of preservation of beta-cell mass in type 2 diabetes, we investigated whether there is the dose-dependent effect of α-LA on pancreatic beta-cells and whether α-LA has a protective role under conditions of oxidative stress in beta-cells.

Materials and methods

Cell culture

INS-1 rat insulinoma cells were cultured in RPMI 1640 (11 mmol/l glucose, 1 mmol/l sodium pyruvate, 10 mmol/l HEPES) supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C and 5% CO_2.

Islet isolations

Pancreatic islets were isolated from male Sprague–Dawley rats (250–300 g) by collagenase digestion and separated by discontinuous gradient purification [26]. Use of the rats for this purpose was approved by the Institutional Experiment and Animal care Committee. Briefly, after collagenase P (1 mg/ml, Roche, Indianapolis, IN) was injected into the bile duct of an anaesthetized rat, swollen pancreas was gently pulled out and then digested at 37°C for 25 min with gentle shaking. After stopping the digestion with cold M199 solution (Gibco, Grand Island, NY), tissue was passed through a 400 mm screen and centrifuged on discontinuous Ficoll gradient purification (Biochrom AG, Berlin, Germany). Islets collected from the interface were washed with M199 solution and individual islets were handpicked using micropipettes. Islets were maintained in Medium 199 with 10% FCS.

Measurement of cell viability

MTT assay. INS-1 cells seeded in 96-well plates were cultured overnight for re-attachment and treated with various concentrations of hydrogen peroxide (Sigma, St. Louis, MO) and/or α-LA for indicated times. α-LA (Thioctacid; MEDA Pharma, Bad Homburg, Germany) was obtained from Bukwang Pharm. Co. (Seoul, Korea). The stock solution of 121 mmol/l α-LA as trometamine salt was added to the culture medium to make different final concentrations. The cells were treated with MTT (0.5 mg/ml) for 4 h at 37°C and then dissolved in DMSO. After 30 min at room temperature, absorbance was measured at 540 nm using a microplate reader (Molecular Devices, Palo Alto, CA). The absorbance of five wells for each treatment was averaged and expressed as a percentage of the mean of control untreated wells.

Acridine orange/propidium iodide (AO/PI) staining. Viability of cells and islets was also assessed by acridine orange/propidium iodide (AO/PI) (Sigma) fluorescent staining. INS-1 cells grown on 8-well chamber slides or islets were stained with 0.67 μmol/l AO and 75 μmol/l PI in PBS for 30 min at 37°C 5% CO_2. After being washed once with PBS, they were examined under a fluorescent microscope. Cells fluorescing green were regarded as viable. Cells fluorescing red, either fully or partially, were regarded as non-viable.

Detection of apoptosis

For detection of phosphatidylserine externalization by flow cytometry, Annexin V-FITC (AV, BD Biosciences, San Diego, CA)/PI assay was used [27]. Briefly, after treatment with reagents (hydrogen peroxide and/or α-LA), INS-1 cells were harvested

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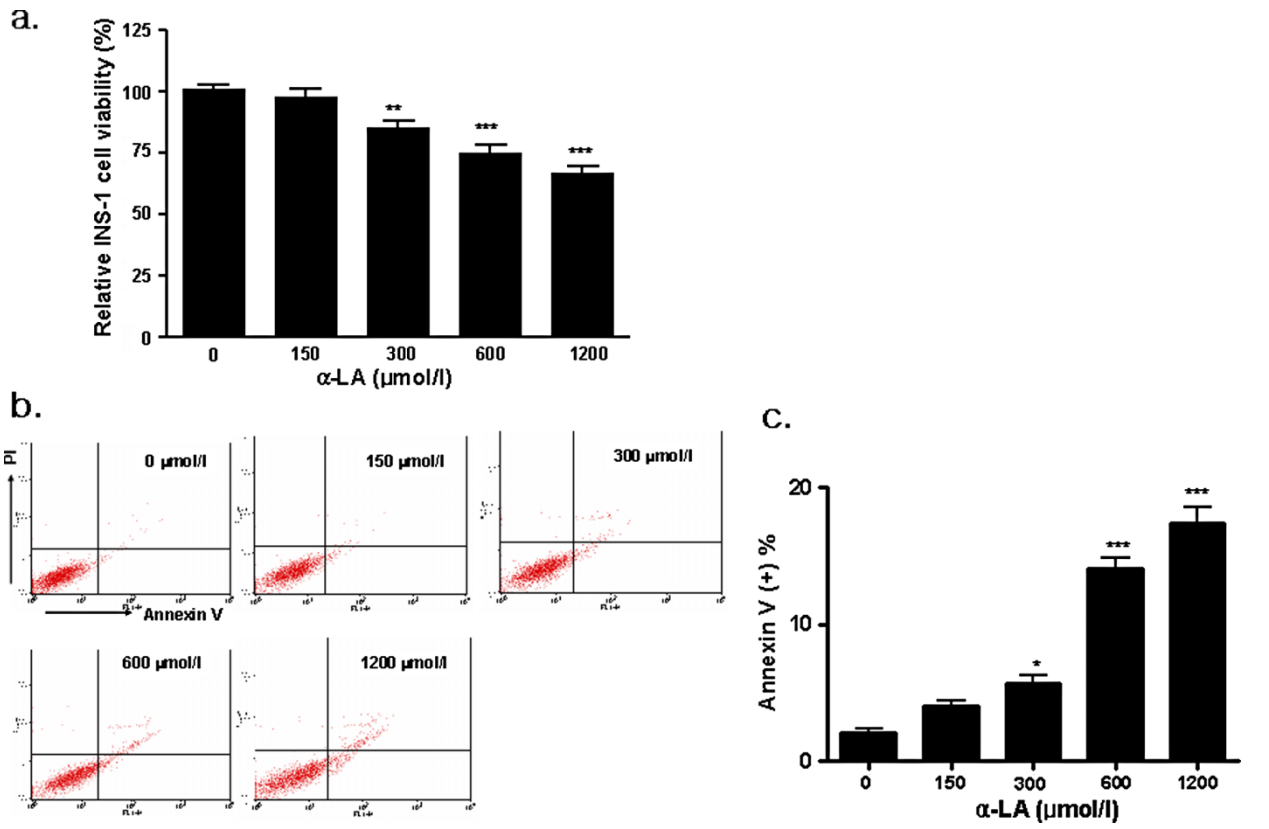

Figure 1. Viability/apoptosis of INS-1 cells after treatment with α-LA. (A) In MTT assay, relative viability (presented as% of untreated control) of INS-1 cells exposed to α-LA for 24 h decreased in a dose-dependent manner: 97.7% with 150 μmol/l, 85.2% with 300 μmol/l, 75.2% with 600 μmol/l and 66.9% with 1200 μmol/l. (B and C) in AV/PI flow cytometry, apoptotic cells were increased dose-dependently with α-LA treatment. The percentage of early and late apoptotic (AV-positive) cells were 2.1% in control, 4.0% with 150 μmol/l, 5.7% with 300 μmol/l, 14.1% with 600 μmol/l and 17.4% with 1200 μmol/l. Results are shown as means ± SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control.

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and washed with ice-cold PBS and then with AV binding buffer (BD Biosciences) and incubated in 100 μl of buffer containing AV and PI for 15 min at room temperature. The cells were immediately analysed by flow cytometry on a BD FACS Calibur (Franklin Lakes, NJ). Cells were considered early apoptotic when they were AV-positive but PI-negative and late apoptotic when they were both AV- and PI-positive.

Determination of reactive oxygen species (ROS) and mitochondrial membrane potential (Δψm)

Intracellular ROS generation was assessed by flow cytometry following staining with 10 μmol/l 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Molecular Probes, Eugene, OR) for 30 min at 37°C [28]. H_2DCFDA is oxidized in the presence of hydrogen peroxide and peroxidases to yield fluorescent 2′,7′-dichloro-fluorescein that can be detected using a 530-nm filter. Values were expressed as a fluorescent cell percentage of total cell counts. Mitochondrial membrane potentials (Δψm) were determined by staining with cationic fluorescent indicator JC-1 (Molecular Probes) [29]. Briefly, INS-1 cells grown on 8-well chamber slides or islets were incubated in culture medium containing 2 μmol/l JC-1 for 30 min at 37°C. The cells or islets were washed in PBS and observed immediately under a fluorescent microscope. JC-1 fluoresces the mitochondria red ($\lambda_{em} = 590$ nm) in healthy cells. During apoptosis, Δψm collapses and the JC-1 cannot accumulate within the mitochondria and remains in the cytoplasm in a green fluorescent monomeric form ($\lambda_{em} = 525$ nm).

Western blot analysis

Protein was extracted with Complete Lysis-M (Roche, Indianapolis, IN) with protease and phosphatase cocktails. Protein samples (50 μg) were separated by electrophoresis through 7–10% polyacrylamide/0.1% SDS gels, transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA) and then immunoblotted. Immunodetection was performed with enhanced chemiluminescent detection technique (Amersham Biosciences, Uppsala, Sweden). Antibodies used in this study were as follows: anti-actin (Sigma), anti-JNK, anti-phospho-JNK, anti-Akt and anti-phospho-Ser473 Akt (Cell Signaling, Boston, MA).

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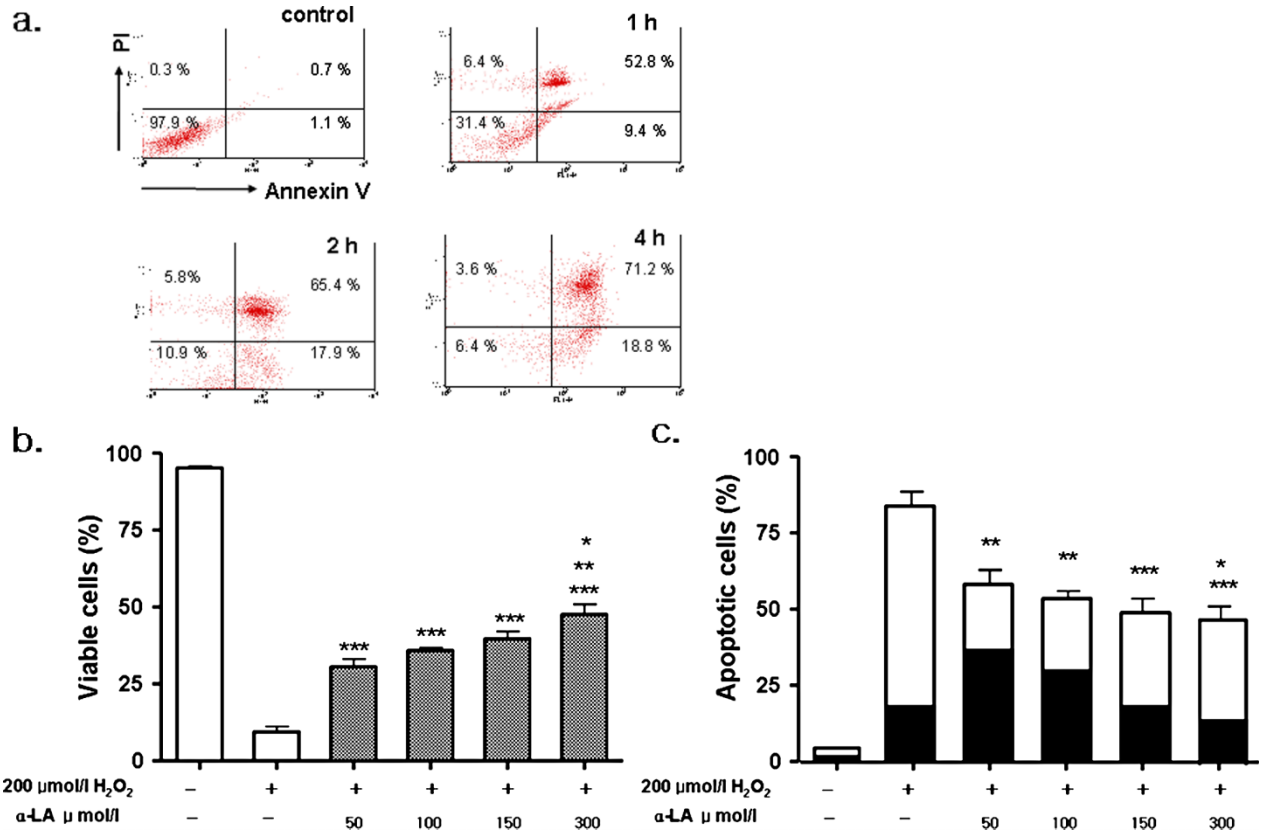

Figure 2. Effect of α-LA on hydrogen peroxide-induced toxicity in INS-1 cells (AV-PI flow cytometry). (A) INS-1 cells exposed to 200 µmol/l hydrogen peroxide for 1–4 h showed apoptotic cell death. (B) Pre-treatment with 50, 100, 150 and 300 µmol/l α-LA for 24 h partially prevented hydrogen peroxide (200 µmol/l for 2 h)-induced INS-1 cell death increasing viable cell% in AV-PI flow cytometry. Results are shown as means ± SD. *** $p < 0.001$ vs only hydrogen peroxide-treated cells. * $p < 0.05$ vs 100 µmol/l α-LA and hydrogen peroxide-treated cells. ** $p < 0.01$ vs 50 µmol/l α-LA and hydrogen peroxide-treated cells. (C) Pre-treatment with α-LA for 24 h reduced the hydrogen peroxide-induced INS-1 cell apoptotic death. Black bar demotes early apoptosis and white bar denotes late apoptosis. ** $p < 0.01$; *** $p < 0.001$ vs only hydrogen peroxide-treated cells. * $p < 0.05$ vs 50 µmol/l α-LA and hydrogen peroxide-treated cells.

Glucose stimulated insulin release in vitro

Static glucose stimulation test was performed with isolated rat islets after overnight culture. Twenty islets were hand picked and incubated in RPMI 1640 medium containing 3.3 mmol/l glucose for a 30-min pre-incubation at 37°C. Thereafter, the islets were incubated sequentially for 30 min each in 2 ml of RPMI 1640 medium containing 3.3 mmol/l glucose (basal), 16.7 mmol/l glucose (stimulatory) and 3.3 mmol/l glucose (recovery). Medium were collected for insulin assay and stored at −20°C. Insulin concentration was measured using an insulin ELISA kit (Shibayagi, Gunma, Japan). The glucose stimulation index (GSI) was calculated as follows: GSI = (2 × stimulatory insulin)/(basal insulin + recovery insulin).

Statistical analysis

Statistical analysis was performed using the PRISM (GraphPad Software Inc, San Diego, CA). Results are expressed as a mean ± SD. The one-way analysis of variance (ANOVA) was used for comparisons involving more than two groups. Statistical significance was defined as the conventional p-value of < 0.05.

Results

Dose-dependent effect of α-lipoic acid on viability of INS-1 cells

Viability of INS-1 cells treated with α-LA (150–1200 µmol/l) for 24 h decreased in a dose-dependent manner in MTT assay (Figure 1A). The percentage of early and late apoptotic (AV- positive) cells was also increased dose-dependently with α-LA treatment in AV-PI flowcytometry (Figure 1B and C). α-LA at a concentration of 150 µmol/l for 24 h did not affect INS-cell viability, and α-LA at or higher than 300 µmol/l induced INS-cell apoptosis and decreased the viability significantly.

Effect of α-LA on hydrogen peroxide-induced toxicity in INS-1 and islet cells

INS-1 cells exposed to 200 µmol/l hydrogen peroxide for 1–4 h showed apoptotic cell death in AV-PI

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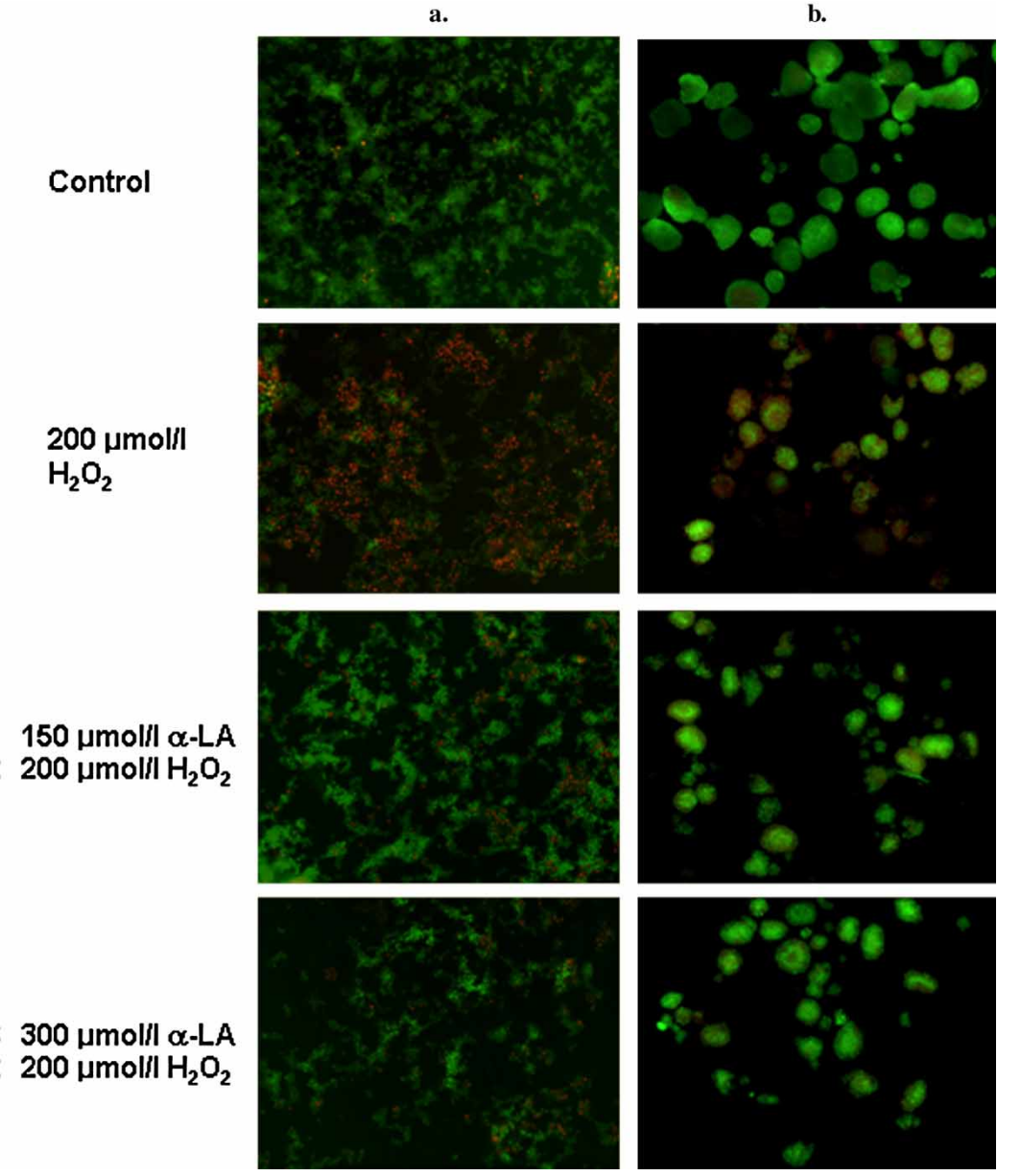

Figure 3. Effect of α-LA on hydrogen peroxide-induced toxicity in INS-1 and rat islet cells (AO/PI staining). (A) Viability of INS-1 cells assessed by AO/PI staining was greatly reduced in hydrogen peroxide (200 µmol/l for 2 h)-treated cells as shown on the large number of red PI-positive cells. The number of red PI-positive cells was reduced with pre-treatment with 150 and 300 µmol/l α–LA for 24 h. (B) Rat islet cell viability decreased after hydrogen peroxide treatment, resulting in increased number of red PI-positive islet cells. Pre-treatment with α-LA reduced this cell damage. Representative images from three experiments are shown. This figure is reproduced in colour in *Free Radical Research* online.

flowcytometry (Figure 2A). Pre-treatment with 50, 100, 150 and 300 µmol/l α-LA for 24 h significantly reduced the hydrogen peroxide-induced INS-1 cell death (Figure 2B). The apoptotic (AV-positive) cells were reduced from 83.4% (hydrogen peroxide-treatment) to 58.4% (50 µmol/l), 53.0% (100 µmol/l), 48.9% (150 µmol/l) and 47.0% (300 µmol/l) with α-LA pretreatment (Figure 2C). Pre-treatment with 600 and 1200 µmol/l α-LA did not reduce the hydrogen peroxide-induced apoptotic cell death at all. For further experiments, 150 and 300 µmol/l α-LA were used to study the cytoprotective effect of α-LA under oxidative stress. In AO/PI staining, pre-treatment with 150 and 300 µmol/l α-LA also significantly reduced the hydrogen peroxide-induced INS-cell death (red PI-positive cells) (Figure 3A). As shown in Figure 3B, rat islet cell viability decreased after 200 µmol/l hydrogen peroxide treatment and pre-treatment with 150 and 300 µmol/l α-LA for 24 h attenuated this toxicity in isolated islets.

Effect of α-LA on intracellular ROS, mitochondrial membrane potential and JNK activation in hydrogen peroxide-treated INS-1 and islet cells

Hydrogen peroxide-treated INS-1 cells (100 µmol/l for 1.5 h) exhibited a significant increase in intracellular ROS in flow cytometry assay with H_2DCFDA staining

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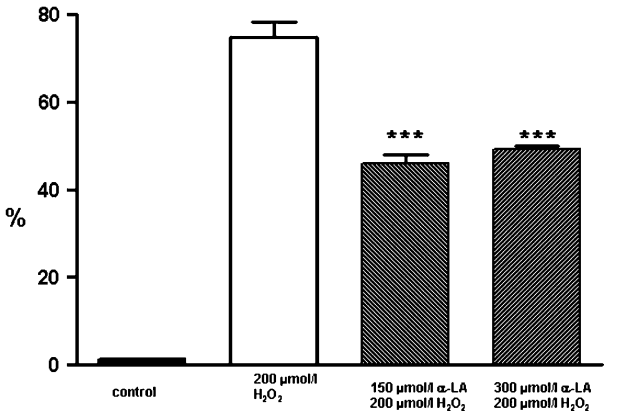

Figure 4. Effect of α-LA on ROS in hydrogen peroxide-treated INS-1 cells. Hydrogen peroxide-treated INS-1 cells (100 μmol/l for 1.5 h) exhibited a significant increase in intracellular ROS in flow cytometry assay with H_2DCFDA staining. ROS levels were attenuated by pre-treatment with 150 and 300 μmol/l α-LA for 24 h. Results are shown as means ± SD. *** $p < 0.001$ vs hydrogen peroxide-treated cells.

(74.7 ± 5.9% vs control 1.4 ± 0.2%, $p < 0.001$, Figure 4). Treatment with 150 and 300 μmol/l α-LA for 24 h did not change intracellular ROS levels significantly compared with control cells. ROS levels after hydrogen peroxide treatment were significantly attenuated by pre-treatment with 150 and 300 μmol/l α-LA (45.9 ± 3.5% and 49.1 ± 1.3, $p < 0.001$ vs hydrogen peroxide-treated, Figure 4). In JC-1 staining, red aggregates were consistently found in the control INS-1 cells, whereas the homogenous green fluorescence was predominant in the 200 μmol/l hydrogen peroxide-treated cells, indicating mitochondrial membrane depolarization and reduced $\Delta\psi m$ (Figure 5A). Pre-treatment with α-LA at 150 and 300 μmol/l attenuated this decrease in $\Delta\psi m$, as reflected by some red aggregates in the INS-1 cells after hydrogen peroxide treatment. The protection from hydrogen peroxide-induced mitochondrial membrane depolarization by α-LA was also observed in isolated rat islets (Figure 5B). As shown in Figure 6, phosphorylation of c-JNK was increased in a response to 2 h exposure to 200 μmol/l hydrogen peroxide and its expression was decreased with pre-treatment with α-LA for 24 h.

Effect of α-LA on PI-3/Akt pathway in INS-1 cells

Compared with untreated INS-1 cells, treatment of INS-1 cells with 150–300 μmol/l α-LA for 30 min led to an increase in phosphorylation of Akt. This α-LA-induced increase in Akt phosphorylation was significantly inhibited by LY294002 (10 μmol/l), indicating they were mediated by the activation of the PI3K pathway (Figure 7A). Interestingly, α-LA elicited a biphasic response, with a peak level of phospho-Akt observed after 10–30 min incubation, followed by a drop at 60 min and then an increase at 24 h of incubation (Figure 7B).

Effect of α-LA on glucose-stimulated insulin release in rat islets

The insulin secreted in response to basal (3.3 mmol/l), stimulatory (16.7 mmol/l) and recovery (3.3 mmol/l) glucose was not significantly different between control and either 150 or 300 μmol/l α-LA-treated rat islets, as shown in Figure 8. The GSI of either 150 or 300 μmol/l α-LA treated rat islets (3.14 ± 0.75 and 2.82 ± 0.55) was also not significantly different from that of control islets (3.05 ± 1.23).

Discussion

In the present study, our attention was focused on the dose-dependent effect of α-LA on pancreatic beta-cell and the potential protective role of α-LA under conditions of oxidative stress in beta-cells at clinically relevant concentrations. First, we observed that the viability of INS-1 cells treated with α-LA (150–1200 μmol/l) for 24 h decreased in a dose-dependent manner. α-LA at or higher than 300 μmol/l induced INS-cell apoptosis and decreased the viability significantly. Our study is consistent with the previously reported findings that α-LA could act as a pro-oxidant resulting in cell damage in chemical systems and living tissues [17,18]. α-LA and DHLA have been shown to stimulate ROS production and MMP depolarization in rat liver mitochondria *in vitro* [20] and to increase NADPH oxidase-induced ROS production and expression of p47phox in rat kidney *in vivo* [21]. Recently, Targonsky et al. [10] reported that in rat islets or MIN6 cells, α-LA at or higher than 500 μmol/l induced AMPK phosphorylation dose-dependently, leading to decreased insulin secretion and beta-cell growth. They also found that 200 μmol/l α-LA acutely increased mitochondrial ROS production and MMP depolarization. Taken together with our observation that α-LA at or higher than 300 μmol/l induced apoptotic cell death in INS-1 cells, α-LA seems to have deleterious effects on otherwise healthy pancreatic beta-cells at certain concentrations.

Oxidative stress is increased under uncontrolled diabetic conditions and is likely involved in progression of pancreatic beta-cell dysfunction found in diabetes [24,25]. Whereas α-LA itself may act as a pro-oxidant in the absence of other pro-oxidants, it has been shown that under conditions of oxidative stress, this compound exhibits beneficial protective properties [21,30]. Does α-LA itself play a beneficial anti-apoptotic role under conditions of oxidative stress in pancreatic beta-cells? To address this question, we used hydrogen peroxide as an oxidative stressor. Exposure to hydrogen peroxide has oxidative stress-mediated toxicity towards beta-cells [31,32]. Our study demonstrated that pre-treatment with 50–300 μmol/l α-LA significantly reduced the hydrogen

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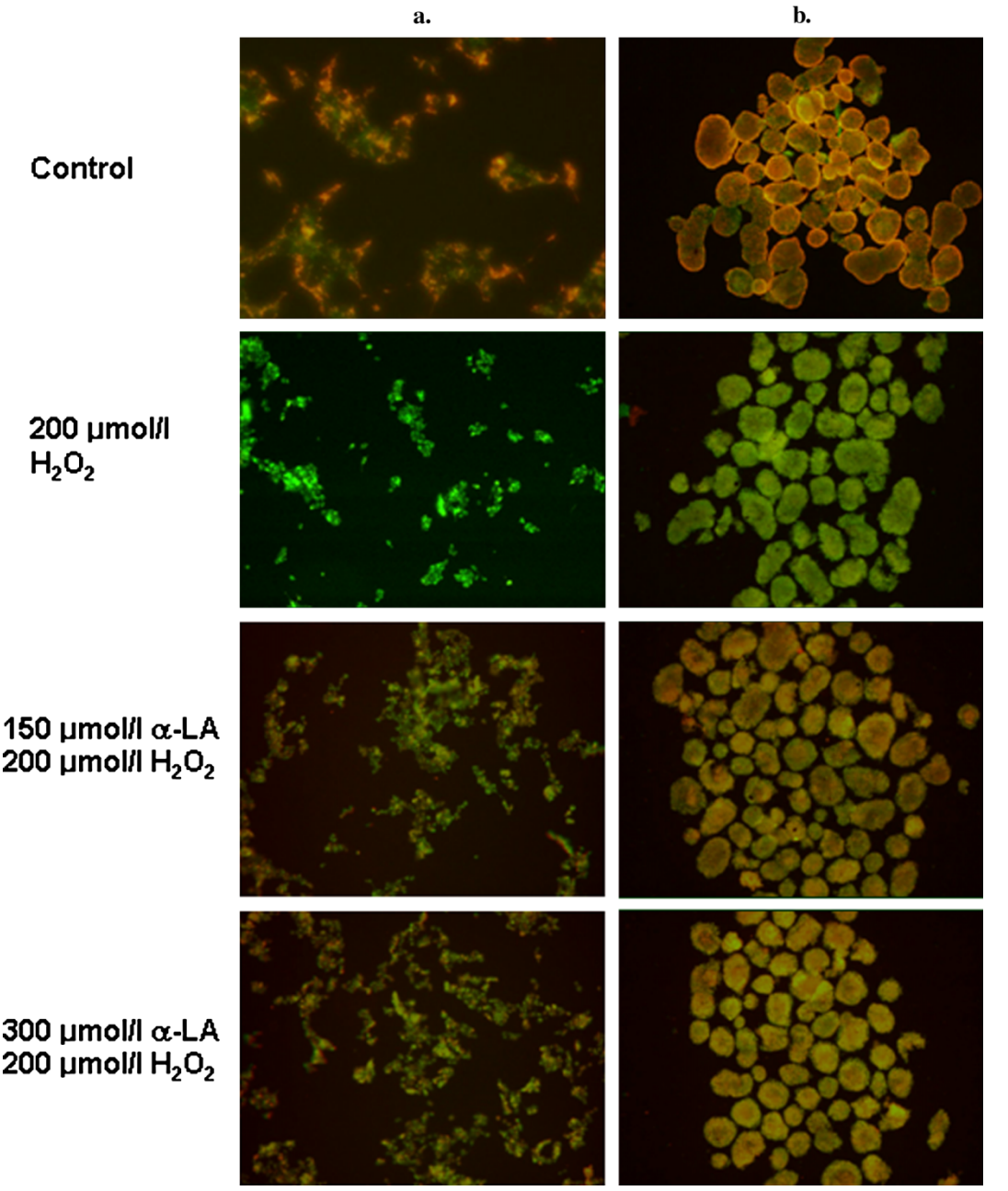

Figure 5. Effect of α-LA on MMP in hydrogen peroxide-treated INS-1 and islet cells. In JC-1 staining, red aggregates were consistently found in the control INS-1 (A) and rat islet (B) cells, whereas the homogenous green fluorescence was predominant in the 200 µmol/l hydrogen peroxide-treated cells, indicating mitochondrial membrane depolarization and reduced Δψm. Pre-treatment with α-LA at 150 and 300 µnmol/l attenuated this decrease in Δψm, as reflected by some red aggregates in the cells after hydrogen peroxide treatment. Representative images from 3–5 experiments are shown. This figure is reproduced in colour in *Free Radical Research* online.

peroxide-induced INS-1 cell apoptotic death. Interestingly, in our results, the treatment of INS-1 cells with 300 µmol/l α-LA in the absence of hydrogen peroxide was associated with a slight but significant INS-cell apoptosis and decreased cell viability. Thus, these findings indicate that, α-LA at certain concentrations may have detrimental effects on beta-cells under normal condition but cytoprotective effects under increased oxidative stress, such as in diabetes.

Concerning diabetic polyneuropathy, the beneficial effects of α-LA seem to be mainly derived from its anti-oxidative properties [4,33]. Our results indicate that the mechanism by which α-LA exerts the anti-apoptotic effect on beta-cells under oxidative stress is via decreasing oxidative stress, as shown in H_2DCFDA flow cytometry. This anti-oxidative effect led to the reduced MMP depolarization and JNK activation. Oxidants have been shown to regulate the JNK activation and oxidant-induced JNK activation has been linked to apoptosis [34]. In concordance with JNK activation in hydrogen peroxide-induced apoptosis in bone marrow stromal cells [35] and lung

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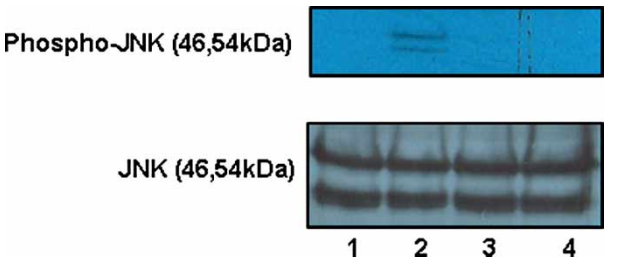

Figure 6. Effect of α-LA on JNK activation in hydrogen peroxide-treated INS-1 cells. Phosphorylation of c-JNK in INS-1 cells was increased in response to 2 h exposure to 200 μmol/l hydrogen peroxide (lane 2) compared with control cells (lane 1) and its expression was decreased with pre-treatment with 150 (lane 3) and 300 (lane 4) μmol/l α-LA for 24 h. Representative immunoblots from three experiments are shown.

fibroblasts [36], we found that hydrogen peroxide activated JNK and α-LA effectively inhibited this activation.

Besides its well-known antioxidant effects, α-LA has recently been reported to exhibit distinct regulatory action on signal-transduction processes related to tissue damage and protection [3,37]. Our results show for the first time that α-LA activates the PI3K/Akt pathway and induces Akt phosphorylation in INS-1 cells. These observations are consistent with previous reports that α-LA activates PI3K/Akt in other cell types [37–40]. The cytoprotective effect of Akt is mainly attributed to its anti-apoptotic potential [41]. Thus, in addition to its well-known antioxidant effect, the activation of the PI3K/Akt pathway might have contributed to the anti-apoptotic effect of α-LA on beta-cells observed in this study.

Our study showed that α-LA may exert both detrimental and cytoprotective effects on pancreatic beta-cells, depending on the underlying pathophysiologic state and on the concentration. A wide range of daily doses of α-LA was used in human trials, ranging from 100–1800 mg/day [42–44] as an oral or intravenous supplementation. α-LA is widely used for the therapy of diabetic polyneuropathy and is well tolerated. The recommended daily dose of α-LA for painful diabetic polyneuropathy is 1200–1800 mg orally or 600 mg per day intravenous infusion for 3 weeks [3]. The oral supplementation of α-LA is absorbed with a time to maximum concentration (t_{max}) of ~ 1 h and exhibits dose proportionality in a dose range of 50–600 mg [12,13]. After a single oral administration of 600 mg α-LA tablets, an individual maximum plasma concentration (C_{max}) was reported to be 17–22 μmol/l. Mean plasma concentration of α-LA in healthy volunteers who received a 35 min infusion of 600 mg α-LA was reported to be 55 μmol/l with SD of 12 μmol/l [15]. Thus, in the current clinical use, α-LA is unlikely to reach a plasma concentration that exerts detrimental effects on beta-cells, such as induction of apoptosis, AMPK

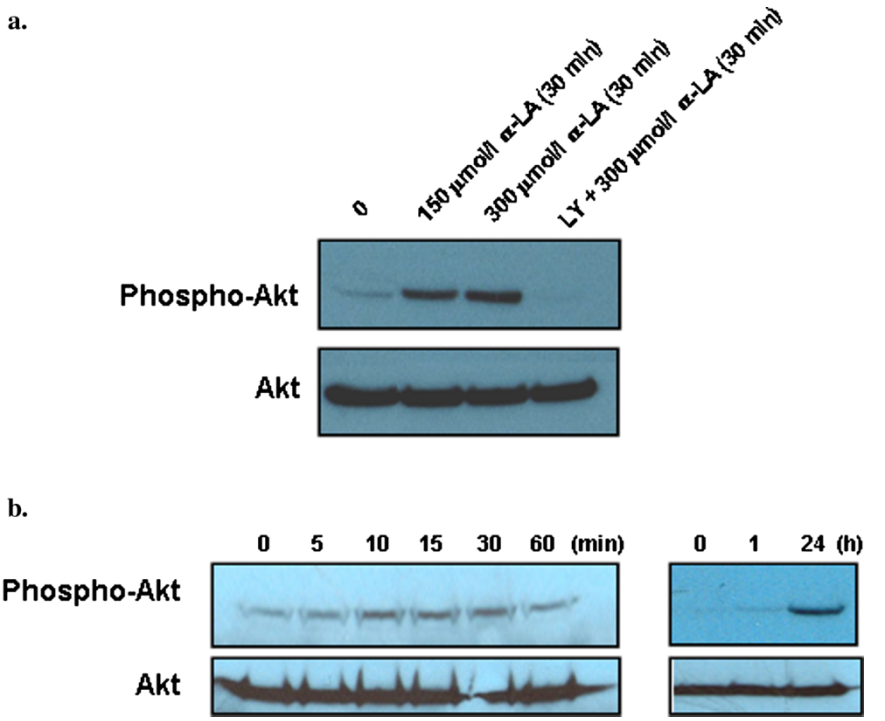

Figure 7. Effect of α-LA on PI-3/Akt pathway in INS-1 cells. (A) Treatment of INS-1 cells with 150 and 300 μmol/l α-LA for 30 min led to an increase in phosphorylation of the Akt. This increase in Akt phosphorylation was inhibited by LY294002 (10 μmol/l). (B) Akt phosphorylation by 300 μmol/l α-LA was biphasic, with a peak level after 10–30 min incubation, followed by a drop at 60 min and then an increase at 24 h. Representative immunoblots from three experiments are shown.

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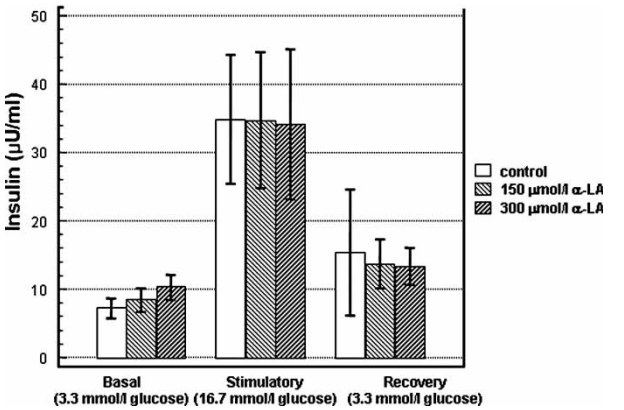

Figure 8. Effect of α-LA on glucose-stimulated insulin release in rat islets. Insulin release in a response to basal (3.3 mmol/l), stimulatory (16.7 mmol/l) and recovery (3.3 mmol/l) glucose was not significantly different between control and either 150 or 300 μmol/l α-LA treated rat islets. Results are shown as means ± SD of four experiments.

activation and ROS-induced impairment of mitochondria, observed in our study and previous studies.

In this study, α-LA at 50–300 μmol/l exerted cytoprotective effects on beta-cells under oxidative stress *in vitro*. *In vivo*, it has been reported that α-LA at a dose of 600 mg/day for 8 weeks in healthy volunteers reduced 8-iso excretion [45]. Another human trial also showed that daily supplementation with 600 mg of α-LA for 3 months can significantly reduce plasma lipid-hydroperoxide formation in diabetic patients [30]. Thus, α-LA at clinically relevant concentrations shows a measurable antioxidant effect and suggests its potential cytoprotective effects on beta-cells. Type 2 diabetes is characterized by a progressive decline in pancreatic islet beta-cell function and mass. The beta-cell loss in type 2 diabetes is accompanied by a marked increase in beta-cell apoptosis, as shown in human pancreas autopsy specimens and in isolated islets [22,23]. Chronic oxidative stress is believed to contribute to glucolipotoxicity and apoptosis of beta-cells [24,25]. In this regard, α-LA is a good antioxidant for consideration as an adjunct therapy in type 2 diabetes.

In summary, we observed that α-LA at or higher than 300 μmol/l acted as a pro-apoptosis inducer in the absence of pro-oxidants in pancreatic beta-cells and at the same time α-LA at 50–300 μmol/l showed an anti-apoptotic effect in the presence of hydrogen peroxide by its antioxidant properties and possibly by Akt phosphorylation. Based on these findings, we conclude that α-LA may exert both pro- and antioxidant effects on beta-cells, depending on the underlying pathophysiologic state and on the concentration. While α-LA is harmful to beta-cells at high concentrations *in vitro*, it may have potential cytoprotective effects on beta-cells in diabetes at clinically relevant concentrations.

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