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Regulation of aging and oxidative stress pathways in aged pancreatic islets using alpha-lipoic acid

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Abstract

Oxidative stress has been involved in the aging process and the pathogenesis of type-2 diabetes, which is a serious health problem worldwide. This study investigates the anti-aging, anti-apoptotic, and antioxidant properties of alpha-lipoic acid (ALA), aiming to improve aged rat pancreatic cells. In this regard, half maximal effective concentration (EC_{50}) of ALA based on the survival of aged pancreatic islet cells was determined as 100 µM. Following this, p38 and p53 genes expression as key factors in aging, oxidative stress biomarkers, insulin secretion, and Pdx1 protein expression were evaluated using real-time PCR, ELISA reader, and fluorescence microscope. It was revealed that ALA reduces and controls the effects of aging on beta cells mainly by suppressing p38 and p53 at the gene level ($P < 0.001$ and $P < 0.01$), respectively, reducing reactive oxygen species ($P < 0.001$) and enhancing levels of thiols ($P < 0.05$) compared with the aged islets. Furthermore, both qualitative and quantitative investigations of insulin secretion have shown that ALA can improve aged cells' function and increase insulin secretion specially in the stimulating concentration of glucose. Also, the expression of Pdx1 was considerably increased by ALA in comparison to the aged pancreatic islets ($P < 0.001$). As far as the authors of the present study are concerned, this is the first study, which evaluated aging associated with p38 and p53 pathways, oxidative stress parameters, and the expression of insulin in beta cells of an aged rat and reaffirmed the fact that ALA has a significant antioxidant role in reducing the aging process.

Keywords Senescence · Diabetes · Oxidative stress · Islet Cells · α-Lipoic acid

Introduction

Cellular aging is a process, which is affected by some factors, resulting in reduced cell survival and reduced physiological activities of tissues and organs. In this process, cells are exposed to various diseases and ultimately lead to cell death [1]. Aging is developed and exacerbated by various factors, including genetic damages, increased DNA methylation, abnormal changes in protein structure, increased reactive oxygen species (ROS), immune system deficiency, and increased inflammatory cytokines [2, 3]. Despite the scientific advances, precise molecular cell mechanisms that affect cellular aging and age-related diseases such as diabetes, cardiovascular disease, cancer, arthritis, osteoporosis, high blood pressure, and Alzheimer's disease are still unknown [4, 5].

One of the most prominent theories of aging is the theory of free radical, first introduced by Harman in the 1950s [6], suggesting that the accumulation of free radicals over

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time can damage macromolecular components and develop pathological disorders, resulting in aging of the cell and the organism [7]. High level of ROS may cause straightforward injury to macromolecules like nucleic acids, lipids, and proteins. As a result of free radicals activity in unsaturated fatty acids, various oxidative products are formed, including lipid peroxide, hydroperoxides, epoxy alcohol, a short chain of malondialdehyde, ethane, and pentane [8], accumulated in biological systems [9]. Then, a wide range of diseases may occur.

Aging is a crucial factor of risk for metabolic illnesses, such as glucose tolerance disorder, obesity, and type-2 diabetes [10, 11]. The prevalence of type-2 diabetes, which is the major cause of health impairment worldwide, rises with age (it is prevalent in the elderly compared to middle-aged adults) and reaches the maximum rate at 60–74 years of age [12–15]. The reduction in the proliferation of beta cells with age may occur by an age-related failure of mitotic signal transduction pathways. p38 MAPK signals can affect CDKI expression in aging islets. The p38 MAPK signals destruction in aging mutant mice reduces the expression of $p16^{INK4a}$, $p19^{arf}$, and other CDKI with a rise in the proliferation of beta cells [16]. This impact may be counterbalanced by the p53-induced phosphatase 1 (WIP1), overexpression of which in middle-aged transgenic mice decreases $p16^{INK4a}$ expression and enhances potential of beta cell regeneration following selective beta cell impairment by streptozotocin [16].

So far, numerous efforts have considered the root cause of aging, and some therapeutic methods have been introduced to stop or at least delay this trend. Increasing the longevity and healthy life is one of the dreams of humankind, and a lot of work has been done to accomplish it [4].

In the other hand, antioxidant compounds can eliminate oxidative stress-related diseases by neutralizing cell-induced damage from free radicals [4, 17]. As a common ingredient in multivitamin formulas, anti-aging supplements, and even pet foods, alpha-lipoic acid (ALA) can be consumed daily due to its importance and positive role in modifying oxidative stress-related diseases [18]. Also, the beneficial effects of ALA, both in the treatment and prevention of diabetes have been evident [19, 20].

Considering the fact that cellular aging is a process associated with oxidative stress and the formation of free radicals in the body and the reality that these factors may affect a series of important cellular pathways associated with cellular function, survival, aging, and cell death, using compounds that reduce the destructive effects of oxidative stress on cells seems essential. In this field, ALA, which plays a major role in reducing oxidative stress and inflammation has therapeutic potential in aging. Here, we used the pancreatic cells of aged rats to evaluate the effects of ALA supplementation, on a battery of islets functions. In this order, the study attempted to provide the anti-aging and anti-diabetic effects of ALA using real-time PCR, ELISA, and immunohistochemistry techniques.

Materials and methods

Chemicals

Rat-specific insulin ELISA kit was purchased from Mercodia (Sweden). Rabbit anti-insulin and mouse anti-Pdx1 from Santacruz biotechnology Inc. (USA), DAPI from Merck Millipore (USA), and Goat Anti-Rabbit IgG H&L (FITC) and Goat Anti-Mouse IgG Fc (DyLight® 650) (ab97018) from Abcam (United Kingdom) were purchased. All the other chemicals that were used in this experiment, such as 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin (BSA), RPMI 1640 medium, HEPES sodium salt, dimethyl sulfoxide (DMSO), DL-dithiothreitol (DTT), and 20,70-dichlorodihydrofluorescein diacetate (DCFH-DA) were obtained from Sigma–Aldrich (GmbH Munich, Germany).

Islets isolation

In this research study, the ethical guidelines of the central committee of Iranian Ministry of Health and Education for research on laboratory animals were followed and the study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS) with code no IR.TUMS.VCR.REC.1395.464. At first, aged rats passed an acceptable time in the lab so that they become adapted to the environment, then they were anesthetized by injection of ketamine-xylazine combined in a ratio of 10:1 (100 mg/kg ketamine/10 mg/kg xylazine) [21]. After laparotomy, the pancreas was removed and washed with buffer Krebs (8 NaCl, 2.38 HEPES, 0.5 glucose·$1H_2O$, 0.42 $NaHPO_4$, 0.27 KCl, 0.22 $CaCl_2{\cdot}2H_2O$, 0.05 $MgCl_2$, in grams per liter, at pH 7.4), so that lymph nodes, fats, and vessels removed from the main tissue. On the next step, tissue and buffer Krebs were put on the ice bath and were cut into small pieces, followed by two times of centrifuge at 3000×*g* for 60 s. For removal of surrounded tissues and preparing separated islets, collagenase enzyme was added to the falcons at 37 °C, and the procedure was then stopped by adding BSA, and then cells were centrifuged for two times in 3000×*g* for 60 s. Using a sampler, islets with a size of 100–150 µm were selected under stereomicroscope. The prepared islets were cultured in standard RPMI 1640 (which contains fetal bovine serum, penicillin–streptomycin and 8.3 mmol/l glucose) and were kept at 37 °C and 5% CO_2 for 24 h [22]. So, they can be used for following experiments.

First phase: safety investigation and finding EC_{50}

MTT

The assay is based on the reduction of MTT. Mitochondrial respiration in viable cells makes the yellow tetrazole change to a purple insoluble formazan. The first step was to incubate cells for 24 h with logarithmic concentrations (1, 10, 100, and 1000 µM) of ALA. They were then centrifuged and buffer phosphate was used to wash the precipitated cells for two times. After adding 50 µL of MTT solution, cells were again incubated for 4 h at 37 °C and 5% CO_2 humidified atmosphere. After adding 150 µL of DMSO solution, absorbance was measured at 570 nm by ELISA reader. The viability of cells is expressed as the percentage of control, which refers to 100 [23]. At the end, the EC50 of ALA on aged Wistar rat pancreatic islets was calculated. In the following, anti-aging, antioxidant and functional effects of ALA were evaluated on aged islets, compared to both young and aged islets without any treatment as negative and positive controls, respectively.

Second phase: function investigation

Insulin secretion assay

After a 24 h of exposing islets to 100 µM ALA, a 24 h of incubation was done, and then 1 mL Krebs medium was added to islets. Centrifuging (3000×*g* for 1 min) was the next step, and after removal of the supernatants, islets were incubated with 2.8 mM glucose for 30 min. In the next step, vials were divided in two groups, to one of them 2.8 mM glucose was added (basal dose) while the other group received 16.7 mM glucose (stimulant dose). After 1 h, the vials were centrifuged and the supernatants were gathered in order to measure insulin secretion by an insulin kit, according to the manufacturer's protocol. The results are reported in micrograms per milligram of protein per hour [21].

Protein assay

Bradford reagent was added to the samples, to measure the total protein concentration of the islets, and after 5 min, the absorbance was read by the spectrophotometer at 595 nm. BSA was used as the standard.

Immunostaining

After 24 h of treatment by ALA, pancreatic islet cells were fixed in 4% formaldehyde for 2 h. The fixed pancreatic islet cells were embedded in paraffin and cut into 7-µm-thick sections. Paraffin-embedded sections were rehydrated, and antigen retrieval was performed using a Thermo Scientific™ PT Module (Greece Co.). The primary antibodies used were rabbit anti-insulin (1:200; sc-9168), and mouse antiPdx1 (1:150; sc-390,792). The secondary antibodies were conjugated to FITC [1:500; goat anti-rabbit IgG (H&L); ab6717; abcam] or DyLight 650 [1:200; Goat Anti-Mouse IgG Fc; ab97018; abcam]. The nuclear counterstain, 4′6′-diamidino-2-phenylindole (DAPI), was also used. All experiments repeated at least two times. The histological slides were evaluated by the independent reviewer, using a fluorescence microscope (Olympus BX51 fluorescence microscope) and were subsequently analyzed using Image-Pro Plus version 6.0 image analysis software (Media Cybernetics, Rockville, MD, USA). Based on the immune-density, the pancreatic islet areas marked with insulin and Pdx1 were selected, and the percentage of positive area for each antibody was reported [24].

Third phase: aging investigation

Real-time reverse transcription polymerase chain reaction (RT-PCR)

In order to study the molecular mechanisms of cell senescence, quantitative RT-PCR was done and the levels of p38 and p53 genes were examined. After washing the cells in sterilized PBS and according to manual guidelines, total RNA was excreted using TRIZol®. The RNA concentration was measured using Thermo Scientific NanoDrop 2000c UV–Vis spectrophotometer (Thermo Scientific, USA). DNase-I and RNase-free kits were used to remove genomic DNA, while cDNA was a reverse transcript by iScript cDNA synthesis kit. As the internal control, primer pairs were selected with glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Quantitative RT-PCR evaluation was done with Light Cycler 96 system (Roche) by SYBR green master mix. Comparative cycle threshold method was used to evaluate the relative gene expression [25]. Table 1 indicates abbreviations, accession number, and sequence of the primers.

Fourth phase: oxidative stress biomarkers investigation

Total thiol molecules (TTM)

As modified in our lab, first 1 mL of Tris–EDTA buffer was added to 50-mL plasma. Then 40 mM ethylene diamine tetra acetic acid, in pH 8.2, was added to 0.4 mL of the samples' supernatants rapidly, followed by a vortex. After that, samples were mixed with 40 mL of 5–5′-dithiobis-2-nitrobenzoic acid (20 mM in pure methanol). Then cells were incubated for a quarter of an hour at room temperature and then a centrifuge was done at 3000×*g* for 15 min. Eventually,

Table 1 Primers of genes p38, p53, and GAPDH for performing RT-PCR

Gene name	Gene symbol	Accession no.	Primer sequence (5′-3′)
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	NM_017008.4	F: AGTCTACTGGCGTCTTCACC R: CCACGATGCCAAAGTTGTCA
Mapk14 mitogen activated protein kinase 14	P38α	NM_031020.2	F: GACACCCCCTGCTTATCTCA R: GACACCCCCTGCTTATCTCA
Tumor protein p53	Tp53	NM_030989.3	F: CCCTGAAGACTGGATAACTG R: AATTAGGTGACCCTGTCGCT

the absorbance was measured at 412 nm and the final results were represented as mM [26].

Reactive oxygen species (ROS)

First, cells were exposed to DTT (50 µM) and lysis buffer, containing EDTA, HEPES, KCl, and sucrose. Then, they were homogenized. In the following, the buffer assay and DCFH-DA was respectively added to the well containing supernatant of the homogenized cells and incubated at 37 °C for 30 min. The measurement of differences between absorbances of the amount of DCF, the fluorescent end product, was then determined using an ELISA fluorimeter (excitation 488 nm; emission 525 nm) through 1 h. The amount of total protein in each well was used to standardize the values. The final results are represented as the percentage of controls, which refers to 100 [27].

Statistic

For each condition investigated in this study (young islets, aged islets, aged islets exposed to ALA), 6 groups of 10 islets were used. At least three independent experiments in repetitions were done. Data are shown as mean ± standard error. One-way ANOVA and Tukey's multi-comparison tests were done for statistical analysis and correlation measurement. StatsDirect software version 3.1.17 was used to determine the statistical differences. The P value of < 0.05 was considered significant.

Results

First phase: safety investigation and finding EC50

MTT

As shown in Fig. 1, all the studied doses of ALA were safe. It also shows that the viability of aged islets differs when they are exposed to different concentrations of ALA. There was a noticeable increment in the percentage of viable cells treated with the 100 µM ALA in comparison to the control group ($P < 0.001$). On the other hand, comparing groups of 10 µM and 1000 µM with the control group, it was seen that the percentage of viable cells have increased in these two groups either ($P < 0.05$).

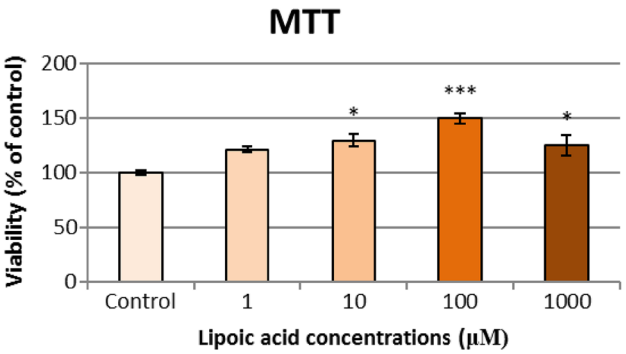

Fig. 1 Effect of different concentrations of alpha-lipoic acid (ALA) on viability of aged rat pancreatic islets after 24 h of exposure. MTT assay was done and EC_{50} of ALA was determined as 100 µM. Data are represented as mean ± standard error of six groups for each condition, with three replicates. $*P < 0.05$; $***P < 0.001$, statistically significant changes when compared with the control group

Second phase: function investigation

Insulin secretion

As shown in Fig. 2, it was seen that in young islets, aged ones, and aged islets receiving ALA, stimulated concentration had caused a significantly higher insulin secretion compared to the basal concentration ($P < 0.001$). The figure also indicates that stimulant and basal concentrations in aged islets made a statistically meaningful lower insulin secretion in comparison to young ones ($P < 0.001$ and $P < 0.01$, respectively). When aged islets were treated with ALA, the insulin secretion related to stimulant concentration was observably lower than the young islets ($P < 0.01$). Another observation showed that when aged islets were exposed to ALA, insulin release in stimulant concentration was adequately higher in comparison to the old islets ($P < 0.01$).

Immunocytochemistry

Post treatment elevation of insulin and Pdx1 expression in isolated pancreatic islets in vitro is represented in Fig. 3. As it was shown, the expression of insulin and Pdx1 was

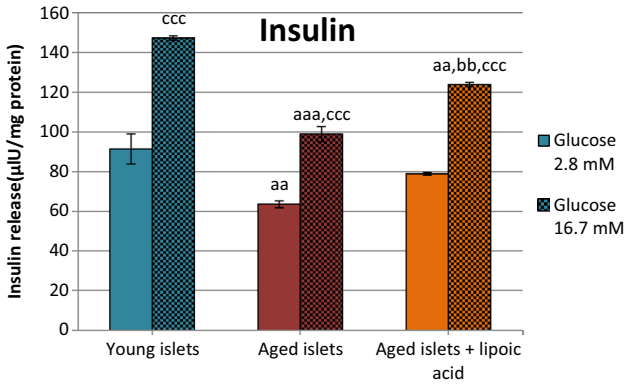

Fig. 2 Effect of 100 µM of alpha-lipoic acid (ALA) on level of insulin secretion in aged rat pancreatic islets after 24 h of exposure. Islets were incubated for 1 h in the presence of 2.8 mM glucose as a basal concentration and 16.7 mM glucose as a stimulant concentration. Data are represented as mean ± standard error of six groups for each condition, with three replicates. $^{aa}P<0.01$ and $^{aaa}P<0.001$, statistically significant changes when compared with the young islets. $^{bb}P<0.01$, statistically significant changes when compared with the aged islets. $^{ccc}P<0.001$, statistically significant changes when basal and stimulated phases were compared

significantly decreased in aged pancreatic islets in comparison to the young islets. After 24-h treatment by ALA, insulin protein expression in the ALA group markedly increased. Although the expression level of insulin did not reach to optimum level as well as young islets, the expression level was significantly increased in comparison to the aged islets ($P<0.01$). Immunostaining method showed that the expression of Pdx1 in aged islets decreased in comparison to the young ones. Pdx1 which are known important for pancreatic development can express in beta-islet cells. The expression level of this marker was also increased at ALA treatments. The expression of Pdx1 was considerably increased by this treatment in comparison to the aged pancreatic islets ($P<0.001$). Overall, it was shown that the expression level of aged pancreatic markers could be reached near the original levels in young islets via ALA treatments.

Third phase: aging investigation

RT-PCR analysis

As it is displayed in Fig. 4a, the relative p38 gene expression is significantly higher in aged islets comparing to the young ones ($P<0.001$). It has to be noticed that when the aged islets were exposed with ALA, the p38 relative expression significantly decreased when compared to the aged islets ($P<0.001$).

Figure 4b also represents that aged islets have much more p53 gene expression than the young islets ($P<0.001$). Moreover, when aged islets were treated with ALA, the p53 relative gene expression was significantly higher than young islets ($P<0.05$) but interestingly, lower than the aged ones ($P<0.01$).

Fourth phase: oxidative stress biomarkers investigation

TTM and ROS

Figure 5a indicates the effect of ALA on oxidative stress biomarkers. As it is visible in the figure, the TTM in the aged islet group has reduced in comparison to young islets ($P<0.001$). In addition, the figure represents that aged islets treated with ALA showed a higher TTM than aged ones ($P<0.05$), while being compared to young islets, the TMM was lower ($P<0.05$).

Furthermore, Fig. 5b shows that ROS in aged islets which were exposed to ALA, were significantly lower than both aged and young islets ($P<0.001$).

Correlation

Correlation analysis between p53 and p38 expression as aging markers, ROS levels as an oxidative biomarker and insulin secretion in the stimulating concentration of glucose (16.7 mM) are shown in Table 2. As it is observed, a statistically significant negative correlation was observed between quantitative analysis of p53 gene expression by real-time PCR and insulin secretion in the stimulant concentration of glucose ($r=-1$, $P<0.01$). In the other hand, no significant correlation was represented between p38 and p53 with ROS ($r=0.616$ and $r=0.891$, respectively).

Discussion

The present study set out with the aim of finding mechanisms on aging-induced disorders in antioxidant system of islets of Langerhans and improving function of the aged islet cells. As a strong relationship between oxidative stress and aging has been reported in the previous literatures, we also showed that ALA can block aging pathways via antioxidant properties in pancreatic islet cells. ALA was selected for this study, among other antioxidants because of having some advantages over others; such as solubility in both aqueous and lipid environments, scavenging free radicals both inside and outside of the cells, having small size, which leads ALA to cross through the plasma membrane and blood–brain barrier, ability to regenerate antioxidants such as vitamin C, etc. These features along with the ability of metal chelating make ALA more powerful than other antioxidants [27].

Cell senescence occurs progressively with age or may occur due to organ premature impairment, which changes

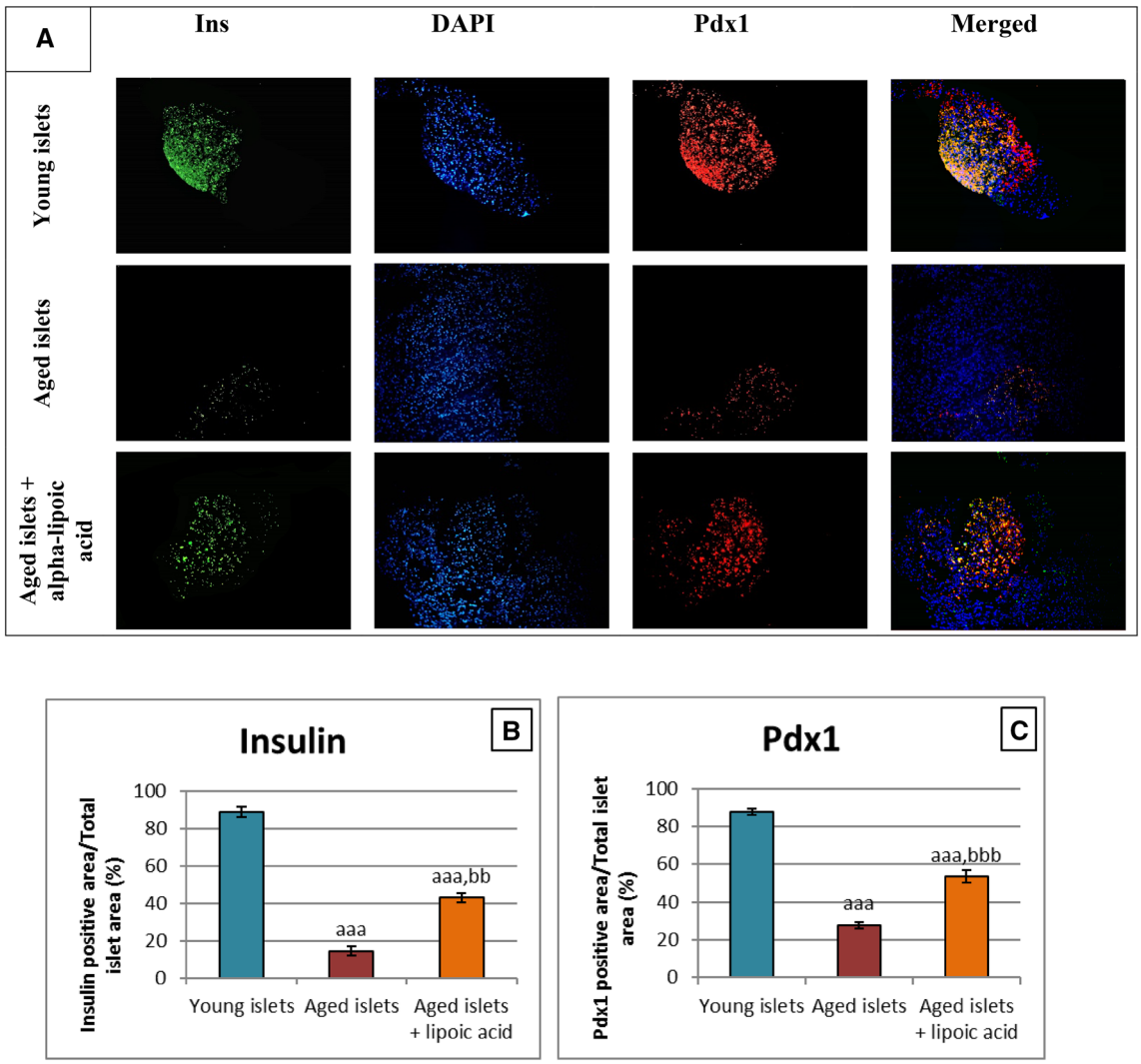

Fig. 3 Endocrine protein expression in young islet cells, aged islet cells, and aged islet cells exposed to alpha-lipoic acid (ALA). **a** Figures of the first column show immunostaining of pancreatic islets against Insulin protein; second column shows DAPI staining as counter-stained; third one shows immunostaining against Pdx1, and the last column is a combination of the previous stainings. All images were taken with X40 objective. **b, c** They show the histograms of the mean fluorescence intensity of Insulin and Pdx1. All results are represented as mean ± standard error of six groups. $^{aaa}P < 0.001$, statistically significant changes when compared with the young islets. $^{bb}P < 0.01$ and $^{bbb}P < 0.001$, statistically significant changes when compared with the aged islets

the function of cell triggered by acute stress. Cellular senescence is an irreparable arrest of cell division which happens following DNA damage, oxidative stress, telomere erosion, or oncogenic activation [28, 29]. The proteins p53, p21, and p16 are inhibitors of cell cycle helping the senescence process. Beta cell proliferation and/or ROS production speed up cellular senescence [30, 31]. It has been reported that larger islets are more frequent in young rats compared with middle-aged rats. Also, beta cell/islet region ratios declined meaningfully with age. These findings show that p53, p21, and p16 senescence markers were overexpressed in pancreatic tissue, and oxidative stress rose by the generation of ROS and down-expression of eNOS in middle-aged rats [32].

Figure 4 of this study indicates the expression of p53 and p38 genes estimated by RT-PCR to determine which aging-related mechanisms are affected by ALA in pancreatic islet cells. While expression of p53 and p38 in aged cells is more than the young ones signed. It can be seen from the data that expression of these genes significantly decreased in pancreatic cells of aged rats exposed to ALA compared to those which did not receive ALA. Furthermore, Table 2 provides the significant negative correlation between p53 expression and the level of insulin secretion.

Proteins, lipids, and DNA face oxidation process under natural physiological status. In some diseases, aging, and due to exposure to some toxic chemicals, the oxidation rate

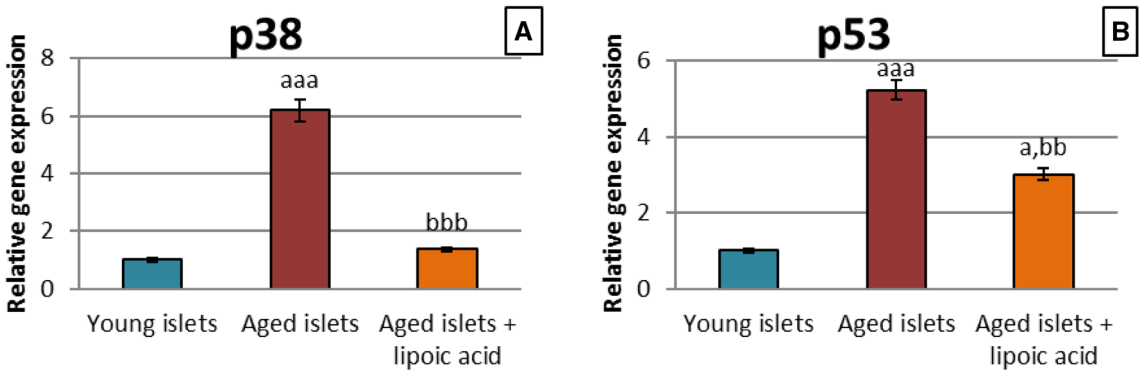

Fig. 4 Description of relative fold changes of genes p38 (**a**) and p53 (**b**) after 24 h exposing aged pancreatic islets to alpha-lipoic acid (ALA) and doing RT-PCR. GAPDH is the housekeeping gene control. Data are represented as mean ± standard error of six groups for each condition, with three replicates. $^{a}P < 0.05$ and $^{aaa}P < 0.001$, statistically significant changes when compared with the young islets. $^{bb}P < 0.01$ and $^{bbb}P < 0.001$, statistically significant changes when compared with the aged islets

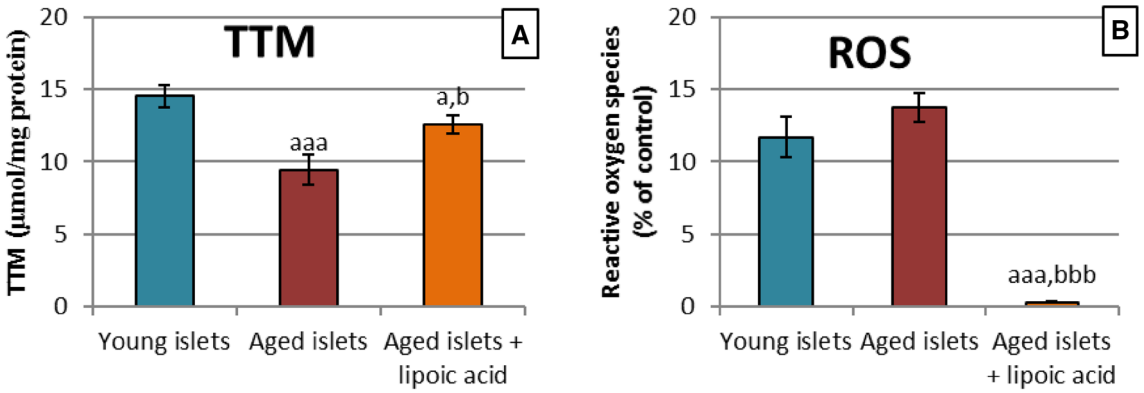

Fig. 5 Effect of alpha-lipoic acid (ALA) on oxidative stress biomarkers in aged rat pancreatic islets. **a** TTM (total thiol molecules) assay and **b** ROS (reactive oxygen species). Data are represented as mean ± standard error of six groups for each condition, with three replicates. $^{a}P < 0.05$ and $^{aaa}P < 0.001$, statistically significant changes when compared with the young islets. $^{b}P < 0.05$ and $^{bbb}P < 0.001$, statistically significant changes when compared with the aged islets

Table 2 Correlation of p53 and p38 gene expression analysis by quantitative real-time PCR, ROS (reactive oxygen species) levels and insulin secretion at stimulated concentration of glucose, in rat pancreatic islets

		Insulin (16.7 mM glucose)	p53	p38
p53	Pearson correlation	−1.000[a]	1	0.908
	Sig. (2-tailed)	0.008		0.275
p38	Pearson correlation	−0.903	0.908	1
	Sig. (2-tailed)	0.283	0.275	
ROS	Pearson correlation	−0.157	0.170	0.567
	Sig. (2-tailed)	0.900	0.891	0.616

[a]Pearson's correlation is significant at the 0.01 level (2-tailed)

of these macromolecules rises. Increased rates of these oxidative stress markers approve the role of oxidative stress in ROS-associated senescence in the pancreas. Pancreas of aging rats has been found to be effective in several biochemical processes, including attenuation of insulin action along with up-regulation of p53 gene. High level of beta-galactosidase may attenuate insulin signaling pathway, possibly leading to insulin resistance as experimentally shown in muscles [33], adipose tissues [34], and in patients with type-2 diabetes [35].

After extraction of pancreatic islets from old and young rats, an obvious difference was seen in the activity of this antioxidant capability of islets. Another crucial determinant of antioxidant system is total thiols, which protect the body against ROS. Total thiols are organic compounds with the sulfhydryl group; reduced levels of total thiols and the resultant oxidative stress bring about numerous chronic diseases, such as diabetes [36, 37]. As far as the authors are

concerned, this is the first study reporting the impact of ALA on antioxidant defense system of the pancreas of aged rats. The antioxidant system of the pancreas is highly susceptible to the toxic metabolites and makes it more impotent to ROS action.

As shown in Fig. 5, following the addition of ALA, a significant increase in the rate of TTM was recorded in the aged islet cells. Interestingly, there was a significant reduction of ROS level in those cells compared to both aged and young islet cells which were not exposed to ALA.

In general, two known factors of aging are oxidative stress and inflammation. Most studies have shown that the induction of oxidative stress through the production of free radicals of ROS accelerates the cellular aging process. There is a balance between the production of oxygen-based compounds and free radicals in the body and antioxidant defense systems; when this balance breaks down in favor of oxygen-based compounds, oxidative stress is created. Free radicals are produced in the body and play an important role in the pathogenesis of diseases and can cause irreparable damage to macromolecules such as DNA, lipids, proteins, and pathways controlling cells, and induce tissue damage [38]. Research has also demonstrated that the anti-proliferative effect of ALA on tumor cells in vitro and in vivo is due to the synergistic effects of antioxidant, apoptosis, anti-proliferative (anti-cellular growth), and anti-inflammatory properties [39].

Generally, aging significantly induces oxidative stress in beta cells, creates ROS with p38, and activates p53. In addition, high expression of p38 was demonstrated by RT-PCR in isolated cells of the old rat. This suggests that the aging of beta cells might be triggered by the activation of p38 and p53. As noted, antioxidants can reduce the aging effect of p53; therefore, ALA can significantly reduce the oxidative stress induced in beta cells. The results indicated that ALA has a positive effect on the weakening of the oxidative stress pathway, which proceeds with increasing the modulation of the beta cell-associated expression of p53 and p38 genes. In other words, it was proved that ALA greatly suppresses the effective parameters contributing to aging in beta cells.

To support our findings more in detail, the activity of insulin and Pdx1 was also measured in beta cells. Moreover, double immunostaining of insulin and Pdx1 in pancreatic islets indicated that exposure of aged rats' islet cells to ALA causes significant activation of Pdx1 leading to a significant increase in insulin secretion. ALA has been proved to mediate its anti-apoptotic action via activation of the insulin receptor/PI3-kinase/Akt pathway. Moreover, it has been determined that ALA has a direct binding site at the insulin receptor tyrosine kinase domain, which may introduce ALA a model substance for the development of insulin mimetic [40]. Figure 2 shows quantitatively that ALA can improve the function of the pancreatic islets via increasing insulin secretion in the aged islet cell; Fig. 3 qualitatively shows this effect.

Though there are many reports indicated that the senescent cell burden rises in the aged tissues; more experiments and research are required on the fundamental mechanisms contributing, in particular, to the early progression of aging in the pancreas of aged subjects and to connect cellular senescence to diabetes pathogenesis [41].

Conclusion

There are remarkable researches on the association of the use of antioxidants in the aging process in islet cells. In this regard, more studies show that the formation of free radicals is one of the most fundamental theories of islet cells aging. Studying the aging parameters in pancreatic cells and the known antioxidant effects of ALA in reducing the negative effects of cellular aging and assessing the survival of cells, oxidative stress markers, and the expression of aging-related genes, it was revealed that ALA reduces and controls the parameters associated with the cell aging in pancreatic cells of old rats. This compound reduces and controls the effects of aging on beta cells mainly by suppressing p38 and p53 at the gene level, reducing ROS, increasing TTM, and affecting insulin secretion and Pdx1 protein expression.

As far as the authors of the present study are concerned, this is the first study, which evaluated aging in beta cells of aged rats and reaffirmed the fact that ALA has a significant antioxidant role in reducing the aging process and it can induce functional protection in the pancreatic islets of aged rats in vitro. Though ALA has the capacity to be evaluated further, both in clinical and experimental studies regarding diabetic toxicity. After conducting in vivo modeling of aging, according to the safety and also existing ALA in human bodies naturally, more work can be undertaken as clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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