Alpha-Lipoic Acid Treatment Reverses 2-Deoxy-D-ribose-Induced Oxidative Damage and Suppression of Insulin Expression in Pancreatic Beta-Cells

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We investigated whether alpha-DL-lipoic acid (LA) could prevent 2-deoxy-D-ribose (dRib)-induced oxidative damage and suppression of insulin expression in pancreatic beta-cells. Stimulation with 50 mM dRib elevated cytotoxicity, apoptosis and intracellular reactive oxygen species (ROS) levels in HIT-T15 cells, but pretreatment with LA significantly reversed the dRib-induced changes. LA directly scavenged hydroxyl radicals generated by a Fenton reaction. Intracellular reduced glutathione (GSH) and oxidized glutathione (GSSG) were depleted by stimulation with dRib but levels were restored by addition of LA to HIT-T15 cells. However, the GSH/GSSG ratio was unchanged by LA treatment. In rat islets, stimulation with 10 mM dRib for 6 h suppressed expression of insulin and pancreatic and duodenal homeobox 1 mRNA and decreased insulin content, but these were dose-dependently reversed when LA was added. Treatment with 1-buthionine-sulfoximine, an inhibitor of intracellular glutathione biosynthesis, completely abolished the protective effects of LA on dRib-mediated glutathione depletion and cytotoxicity in HIT-T15 cells. In summary, LA reversed the dRib-induced oxidative damage and suppression of insulin expression in beta-cells. Enhanced intracellular total glutathione production, rather than the scavenging of ROS, is possibly the mechanism for the protective effect of LA.

Key words alpha-DL-lipoic acid; 2-deoxy-D-ribose; oxidative stress; pancreatic beta-cell; glutathione

High-glucose-induced oxidative stress is one of the important causes of progressive beta-cell derangement in individuals with type 2 diabetes. In vitro, glucose takes a long time to produce oxidative damage in beta-cells because it is the least reactive of the sugars. However, 2-deoxy-D-ribose (dRib), a strongly reducing sugar, promptly induces oxidative stress and apoptosis in beta-cell lines. The use of dRib has made oxidative stress research easier and more effective in beta-cells.

Alpha-lipoic acid (LA) is a naturally occurring organosulfur compound that acts as a cofactor in mitochondrial respiratory enzymes and has strong antioxidant effects. LA is currently used to treat diabetic polyneuropathies and it has shown efficacy of LA is mostly attributable to its unique antioxidant properties.

In this study, we examined whether treatment with LA could prevent dRib-induced oxidative damage and suppression of insulin expression in beta-cells. We also investigated how LA treatment reversed the oxidative stress caused by dRib.

MATERIALS AND METHODS

Materials LA, buthionine sulfoximine (BSO), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), dihydrorhodamine 123 (DHR 123), Ficoll, dimethylsulfoxide (DMSO), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), FeSO4 and H2O2 were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). dRib and N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (HEPES) were obtained from Amresco (Solon, OH, U.S.A.). Hydrochloric acid and ethanol were from Merck (Darmstadt, Germany). Roswell Park Memorial Institute (RPMI)-1640 medium, Dulbecco’s phosphate buffered saline (DBPS), trypsin, penicillin, streptomycin and TRIzol reagent were purchased from Gibco Invitrogen (Grand Island, NY, U.S.A.). Collagenase P and Hank’s balanced salt solution (HBSS) were from Roche Applied Science (Mannheim, Germany). Fetal bovine serum (FBS) was from HyClone (Logan, UT, U.S.A.). All culture dishes were from BD Falcon (Franklin Lakes, NJ, U.S.A.).

Cell Culture Insulin-secretion HIT-T15 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells were cultured under 5% CO2 and 95% humidified air at 37°C in RPMI-1640 medium supplemented with 10% FBS, 100 mM penicillin and 100 mg/mL streptomycin. They were separated and subcultured by trypsinization when they reached about 70% confluence. Two days after subculture, the culture medium was replaced with fresh RPMI-1640 containing 10% FBS and 35 or 50 mM dRib was added to the medium after pretreatment with various concentrations of LA for 30 min. The cultures were then incubated for 6 or 24 h.

Islet Isolation and Culture Rat islets were isolated as described by Lacy and Kostianovsky. Six- to eight-week-old Sprague Dawley rats were obtained from OrientBio Corp. (Seongnam City, Gyeonggi, South Korea). Rats were killed by cervical dislocation and the abdomen was opened; then 9–10 mL of collagenase P (1 mg/mL in HBSS) was injected into the pancreas via the bile duct. The distended pancreas was removed for digestion at 37°C for about 12 min and tissues were separated on a Ficoll gradient. Isolated islets were suspended in RPMI-1640 medium supplemented with 20%
FBS and 11.1 mM glucose for 24 h before experiments. Thereafter, the medium was replaced by fresh RPMI-1640 containing 10% FBS, and 10 mM dRib was added to the medium after pretreatment with various concentrations of LA for 30 min. The cultures were incubated for 6 h.

This study was approved by the Institutional Animal Care and Use Committee of Jeju National University.

Assessment of Cell Viability The MTT assays to evaluate cell viability were performed as described.4,5

Flow Cytometry for Measuring Apoptosis Flow cytometry with annexin V and propidium iodide (PI) double staining was done as described previously.4,6 Briefly, HIT-T15 cells were cultured in 6-well plates at a density of $5 \times 10^5$ per well. The cells were stimulated with 50 mM dRib and LA for 24 h. The stained cells were analyzed using a fluorescence-activated cell sorter (FACSCalibur, BD Bioscience, San Jose, CA, U.S.A.) and quadrant data were calculated with CellQuest software (BD Bioscience). Ten thousand cells were evaluated for each sample.

Assessment of Intracellular Reactive Oxygen Species (ROS) Levels Intracellular ROS levels were assessed using the fluorescein-labeled dye DHR 123 as described previously.4 Intracellular ROS levels were assessed using a fluorescein-labeled dye DHR 123 as described previously.4 Intracellular ROS levels were assessed using a fluorescein-labeled dye DHR 123 as described previously.4 Intracellular ROS levels were assessed using a fluorescein-labeled dye DHR 123 as described previously.4 Intracellular ROS levels were assessed using a fluorescein-labeled dye DHR 123 as described previously.4

Assessment of Hydroxyl Radical-Scavenging Activity ESR spectroscopy was performed for evaluating hydroxyl radical-scavenging activity in a cell-free system. Hydroxyl radicals were generated by the Fenton reaction and reacted with a DMSO-treated control group.

Measurement of Intracellular Glutathione Levels Intracellular reduced glutathione (GSH) and oxidized glutathione (GSGG) were measured using a Glutathione assay kit (Cayman Chemical Company, Ann Arbor, MI, U.S.A.) based on an enzymatic recycling method using glutathione reductase. HIT-T15 cells were cultured in 6-well culture plates at a density of $5 \times 10^5$ per well. The cells were preincubated with LA for 30 min and then cultured with $30 \mu M$ dRib for 6 h. Cells were washed with ice-cold DPBS and sonicated, followed by centrifugation at 10000 $\times g$ for 15 min. The resulting supernatants were used immediately for measurement of GSH and GSGG. The amount of total cellular protein in the sample was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.).

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction (PCR) Rat islets were preincubated with LA for 30 min at various concentrations (see below) and then cultured with 10 mM dRib for 6 h. They were stimulated with lower concentrations of dRib for a shorter time than in the other experiments because cell injury could interfere with mRNA and protein measurements. Total RNA was isolated from cultured islets using TRIzol (Gibco Invitrogen) according to the manufacturer’s instructions. RNA isolation was carried out in an RNase-free environment. Then, 4 $\mu$g aliquots of RNA were reverse transcribed using MuLV reverse transcriptase (Promega, Madison, WI, U.S.A.), oligo(dT)15 primer, deoxyribonucleotide triphosphate (dNTP) (0.5 $\mu$m) and 1 U RNase inhibitor. Quantitative real-time PCR was performed using 2X SYBR Green PCR master mix (Bio-Rad, Hercules, CA, U.S.A.) and an iQ5T 5 multicolor real-time PCR detection system (Bio-Rad). PCR was performed to amplify synthesized cDNA in the presence of specific primers, for 40 cycles at 95°C for 20 s, 60°C for 20 s and 72°C for 30 s, with an initial cycle of 95°C for 5 min. PCR reactions were carried out using specific primers for the cDNA sequences for insulin (forward 5'-GAA CGG CTA CAA TCT CCG AGG GCA'-3'), reverse 5'-TTCC AGGAG CCG GTT GTCAT CACA A-3'), pancreatic and duodenal homeobox 1 (PDX-1) (forward 5'-AGT TTG CAG GTG CTT CGG GAA and reverse 5'-TACC AC GG TGA GCT TGG CTG-3'), and β-actin (forward 5'-TTCC TGG CCT CAC TG CCA C-3' and reverse 5'-GG GCC GAG CACT CA TCG TAC T-3').

Measurement of Insulin Content Isolated islets were preincubated with 0.1, 0.5 or 1 mM LA for 30 min and then stimulated with 10 mM dRib for 6 h. Then, the cells were harvested and washed twice with cold DPBS. The cells were lysed in a lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 1 mM NaVO₄, 10 mM NaF, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 25 μg/mL aprotinin, 25 μg/mL leupeptin, and 1% Nonidet P-40) to obtain whole cell protein and kept on ice for 30 min. The cell lysates were centrifuged at 15000 $\times g$ at 4°C for 15 min. The supernatant was used for determining the insulin and protein contents. Insulin was determined using an enzyme-linked immunosorbent assay (ELISA) kit with rat insulin as standard, according to the manufacturer’s instructions (Mercodia, Uppsala, Sweden). Protein content was measured using a BCA protein assay reagent kit (Pierce). The intracellular insulin content was corrected for the total protein content and expressed as μg/mg protein.

Statistical Analysis All data are expressed as the mean± standard error (S.E.). Data from multiple groups were assessed by one-way analysis of variance (ANOVA), followed by Duncan’s post hoc test. All analyses were performed using SPSS software (version 14.0; SPSS Inc., Chicago, IL, U.S.A.) and $p<0.05$ was considered significant.

RESULTS

LA Attenuated dRib-Induced Oxidative Damage in HIT-T15 Cells To assess the effects of LA on the reduction of cell death caused by dRib, we conducted MTT cell viabil-
ity assays and flow cytometry with annexin V and propidium iodide (PI) double staining. Stimulation with 35 mM dRib for 24 h profoundly decreased HIT-T15 cell viability, but the addition of LA prevented this dRib-induced cytotoxicity in a dose-dependent manner (Fig. 1). Flow cytometry showed that 50 mM dRib stimulation noticeably increased the proportions of apoptotic and necrotic cells (annexin V+/PI– and annexin V+/PI+ populations, respectively) and pretreatment with 1 mM LA significantly reversed the apoptosis and necrosis caused by dRib (Table 1 and Supplementary Fig. 1). Intracellular reactive oxygen species (ROS) production was measured using flow cytometry with DHR 123 staining. Exposure of HIT-T15 cells to 50 mM dRib for 6 h markedly increased the ROS level, which was partially prevented by pretreatment with 1 mM LA (Table 2 and Supplementary Fig. 2). In addition, we performed ESR analysis to determine whether LA could scavenge ROS directly in a cell and dRib-free system. Pretreatment with LA dose-dependently reduced the amount of hydroxyl radicals, the most powerful ROS, generated in a Fenton reaction system (Figs. 2A, B).

Effects of dRib and LA on Insulin mRNA and Content

Fig. 1. Effect of LA Treatment on dRib-Mediated Cytotoxicity
HIT-T15 cells were preincubated with LA for 30 min at the indicated concentrations and then cultured with 35 mM dRib for 24 h. Cell viability was determined by an MTT assay. Data are expressed as the mean±S.E. of the percentage of viable cells relative to the untreated control. This experiment was performed twice, in quadruplicate. **p<0.01 vs. vehicle (0.1% DMSO)-treated control; †p<0.05 and ††p<0.01 vs. 35 mM dRib alone by one-way ANOVA with Duncan’s post hoc test.

Fig. 2. Scavenging Effect of LA Treatment on Hydroxyl Radical Generation
(A) Representative ESR spectra of DMPO–hydroxyl radical conjugates measured in the Fenton reaction. (B) Comparison of hydroxyl radical-scavenging activity between various concentrations of LA in the reaction as in (A). Reaction mixtures contained 60 mM DMPO, 2 mM FeSO4, and 2 mM H2O2 in DPBS with and without various concentrations of LA dissolved in 0.1% DMSO. The results are the mean±S.E. from four independent experiments. **p<0.01 vs. vehicle (0.1% DMSO)-treated control; †p<0.05 and ††p<0.01 vs. 35 mM dRib alone by one-way ANOVA with Duncan’s post hoc test.
To test whether the effects of dRib and LA on HIT-T15 cells were applicable to primary pancreatic β-cells, we isolated and cultured islets from rats. Changes in insulin and PDX-1 mRNA and insulin contents were assessed using quantitative PCR and ELISA, respectively. Stimulation with 10 mM dRib for 6 h suppressed the insulin mRNA expression and the addition of 0.1, 0.5 or 1.0 mM LA significantly inhibited decreases in insulin mRNA level in a dose-dependent manner (Fig. 3A). 2-Deoxy-D-ribose- and LA-induced changes in mRNA expression for PDX-1, an important transcription factor for the normal function and development of pancreatic islets, were similar to the changes in mRNA expression for insulin (Fig. 3B). Islet insulin content was also decreased by the dRib stimulation, but this decrease was completely reversed by pretreatment with various concentrations of LA (Fig. 3C).

**LA Treatment Increased the Total Glutathione Pool in HIT-T15 Cells** Glutathione has a major role in the protection of cells against oxidative stress. Therefore, we assessed the effects of dRib and LA on glutathione homeostasis in HIT-T15 cells. Stimulation with 35 mM dRib for 6 h suppressed the insulin mRNA expression and the addition of 0.1, 0.5 or 1.0 mM LA significantly inhibited decreases in insulin mRNA level in a dose-dependent manner (Fig. 3A). 2-Deoxy-d-ribose- and LA-induced changes in mRNA expression for PDX-1, an important transcription factor for the normal function and development of pancreatic islets, were similar to the changes in mRNA expression for insulin (Fig. 3B). Islet insulin content was also decreased by the dRib stimulation, but this decrease was completely reversed by pretreatment with various concentrations of LA (Fig. 3C).

Table 1. Percentages of Annexin V-Positive Cells in Vehicle- and dRib-Treated HIT-T15 Cells, with or without LA Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Annexin V-positive cells (%)</th>
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<tbody>
<tr>
<td>Vehicle (0.1% DMSO)</td>
<td>8.5±4.4</td>
</tr>
<tr>
<td>50 mM dRib alone</td>
<td>79.0±5.4**</td>
</tr>
<tr>
<td>1.0 mM LA + 50 mM dRib</td>
<td>31.5±7.4**</td>
</tr>
</tbody>
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Grouped data from the experiments illustrated in Fig. 2 are expressed as the mean±S.E. of four independent experiments. **p<0.01 vs. control (0.1% DMSO) and ††p<0.01 vs. 50 mM dRib group evaluated by one-way ANOVA with Duncan’s post hoc test.

Table 2. Relative Intracellular ROS Levels in Vehicle- and dRib-Treated HIT-T15 Cells, with or without LA Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative fluorescence (fold change from the vehicle-treated group)</th>
</tr>
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<tr>
<td>Vehicle (0.1% DMSO)</td>
<td>1.0</td>
</tr>
<tr>
<td>50 mM dRib alone</td>
<td>67.5±8.0**</td>
</tr>
<tr>
<td>1.0 mM LA + 50 mM dRib</td>
<td>15.9±3.3**</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±S.E. of four independent experiments. **p<0.01 vs. control (0.1% DMSO) and ††p<0.01 vs. 50 mM dRib alone evaluated by one-way ANOVA with Duncan’s post hoc test.

**in Rat Islets** To test whether the effects of dRib and LA on HIT-T15 cells were applicable to primary pancreatic β-cells, we isolated and cultured islets from rats. Changes in insulin and PDX-1 mRNA and insulin contents were assessed using quantitative PCR and ELISA, respectively. Stimulation with 10 mM dRib for 6 h suppressed the insulin mRNA expression and the addition of 0.1, 0.5 or 1.0 mM LA significantly inhibited decreases in insulin mRNA level in a dose-dependent manner (Fig. 3A). 2-Deoxy-d-ribose- and LA-induced changes in mRNA expression for PDX-1, an important transcription factor for the normal function and development of pancreatic islets, were similar to the changes in mRNA expression for insulin (Fig. 3B). Islet insulin content was also decreased by the dRib stimulation, but this decrease was completely reversed by pretreatment with various concentrations of LA (Fig. 3C).

**LA Treatment Increased the Total Glutathione Pool in HIT-T15 Cells** Glutathione has a major role in the protection of cells against oxidative stress. Therefore, we assessed the effects of dRib and LA on glutathione homeostasis in HIT-T15 cells. Stimulation with 35 mM dRib for 6 h suppressed the insulin mRNA expression and the addition of 0.1, 0.5 or 1.0 mM LA significantly inhibited decreases in insulin mRNA level in a dose-dependent manner (Fig. 3A). 2-Deoxy-d-ribose- and LA-induced changes in mRNA expression for PDX-1, an important transcription factor for the normal function and development of pancreatic islets, were similar to the changes in mRNA expression for insulin (Fig. 3B). Islet insulin content was also decreased by the dRib stimulation, but this decrease was completely reversed by pretreatment with various concentrations of LA (Fig. 3C).

**Treatment with BSO Abolished the Protective Effects of LA on dRib-Mediated Glutathione Depletion and Cytotoxicity** We sought to assess whether an increased total glutathione pool was associated with an increased synthesis of GSH. HIT-T15 cells were treated with 200 µM BSO, a specific and irreversible inhibitor of glutamate cysteine ligase...
Fig. 4. Effects of LA Treatment on dRib-Induced Depletion of Intracellular GSH and GSSG Contents

HIT-T15 cells were preincubated with 0.1, 0.5 or 1.0 mM LA for 30 min and then cultured with 35 mM dRib for 6 h. The intracellular glutathione concentrations were measured using a glutathione assay kit. This experiment was performed twice, in quadruplicate. **p<0.01 vs. vehicle (0.1% DMSO)-treated control; ††p<0.01 vs. 35 mM dRib alone by one-way ANOVA with Duncan’s post hoc test.

(GCL)—a rate-limiting enzyme for GSH biosynthesis—prior to treatment with 35 mM dRib and 1 mM LA. As shown in Fig. 5A, pretreatment with 200 μM BSO completely abolished the preventive effect of 1 mM LA on dRib-induced GSH depletion. Moreover, LA treatment did not attenuate the BSO-mediated depletion of intracellular glutathione (Fig. 5A). The protective effect of LA on dRib-induced cytotoxicity was also reversed by pretreatment with BSO (Fig. 5B). These results suggest that LA attenuates the dRib-induced oxidative damage by augmenting intracellular GSH synthesis. Pretreatment with BSO alone significantly decreased the amount of GSH, but no more than did treatment with dRib alone (Fig. 5A). However, in the MTT viability assay, the cells treated with BSO alone did not show any survival difference compared with the vehicle-treated control group (Fig. 5B). Therefore, it seems that different mechanisms underlie the BSO- and dRib-induced reductions in intracellular glutathione content.

DISCUSSION

In this study, we found that LA treatment prevented dRib-induced oxidative damage and suppression of insulin expression in HIT-T15 cells and rat islets, respectively. Other authors have reported that LA protected β-cell lines from oxidative damage. However, to our knowledge, it has never been reported that LA treatment could preserve insulin expression in isolated islets. We also found that LA increased the total intracellular content of glutathiones in HIT-T15 cells. Lee et al. reported that LA treatment protected β-cells against hydrogen peroxide-induced oxidative stress and Yang et al. found that it inhibited high-glucose-induced apoptosis in HIT-T15 cells. However, they did not investigate the relationship between LA treatment and intracellular glutathione. These aspects make our research different from previous β-cell studies using LA.

The method of action of LA as an antioxidant is known to be diverse. Direct scavenging of ROS, regeneration of endogenous antioxidants (e.g., GSH and vitamin E) and metal chelation are the best-known mechanisms. Increased production of intracellular glutathione was found to be another antioxidant mechanism of LA. In our study, LA treatment scavenged hydroxyl radicals directly and reversed the dRib-induced depletion of total glutathione. It increased both intracellular GSH and GSSG levels but the GSH/GSSG ratio was not changed significantly. This means that LA treatment did not modulate the redox potential of glutathiones; rather, it elevated the total concentration of intracellular glutathiones. Thus, the protective effect of LA might not be attributable to the scavenging of ROS. Considering our measures of the GSH/GSSG ratio, antioxidant regeneration and metal chelation might also not contribute to the protective mechanism of LA. However, we showed that BSO, a specific inhibitor of rate-limiting enzyme in GSH biosynthesis, completely eliminated the protective effect of LA treatment on dRib-induced GSH decrement and cytotoxicity. This suggests that increasing GSH biosynthesis is the most likely mechanism in the protective effect of LA treatment on dRib-induced oxidative damage. Previous reports described how exogenous LA treatments increased glutathione synthesis in cell and animal models. Suh et al. reported that injections of LA attenuated the age-related loss of GSH synthesis by increasing GCL expression and activity in the rat liver. However, others asserted that LA treatment increased intracellular GSH synthesis through a different mechanism. Han et al. found that LA treatment increased the de novo synthesis of cellular glutathione by improving cystine utilization, and not by increasing GCL expression or activity. In our study, LA treatment might have increased the GCL expression/activity or provided a substrate for intracellular GSH synthesis, but we did not perform experiments to distinguish those mechanisms.

Our research team has been trying to elucidate the specific mechanism of the dRib-induced oxidative damage in β-cells. We previously reported that dRib treatment gave rise to oxidative stress and apoptosis in HIT-T15 cells and that the restoration of intracellular GSH levels was a prerequisite for protecting the cells against dRib-induced oxidative damage. However, we still do not understand the mechanism of action of dRib. Here, we showed that dRib stimulation lowered the intracellular GSH level to a similar degree to treatment with BSO alone but increased the rate of cell death more than did BSO (Fig. 5). Therefore, we speculate that dRib does not simply inhibit GCL action and that its mechanism differs significantly from that of BSO. Further research will aid iden-
for 6 h. The intracellular glutathione concentrations were measured using a glutathione assay kit. (B) Cells were preincubated with 200 µM BSO for 30 min, and then 1.0 mM LA was added to the medium containing 35 mM dRib. The cultures were incubated for 24 h. Cytotoxicity was assessed by an MTT method. These experiments were performed twice, in quadruplicate. Data with different letters are significantly different (p<0.05) according to one-way ANOVA with Duncan’s post hoc test (p<0.05).

Fig. 5. Effects of BSO on the Protective Effects of LA Treatment on dRib-Mediated Glutathione Depletion (A) and Cytotoxicity (B) in HIT-T15 Cells

(A) Cells were preincubated with 200 µM BSO for 30 min, and then 1.0 mM LA was added to the medium containing 35 mM dRib. The cultures were incubated for 6 h. The intracellular glutathione concentrations were measured using a glutathione assay kit. (B) Cells were preincubated with 200 µM BSO for 30 min, and then 1.0 mM LA was added to the medium containing 35 mM dRib. The cultures were incubated for 24 h. Cytotoxicity was assessed by an MTT method. These experiments were performed twice, in quadruplicate. Data with different letters are significantly different (p<0.05) according to one-way ANOVA with Duncan’s post hoc test (p<0.05).

tification of the exact mechanism of dRib-induced oxidative damage.

Glucose toxicity is defined as irreversible β-cell failure caused by chronic exposure to high-glucose environments. It is considered to be caused by increased oxidative stress. Glucose toxicity involves decreases in insulin synthesis and secretion and increase in apoptosis in pancreatic β-cells. Decreased insulin gene expression is largely attributable to post-transcriptional loss of PDX-1 mRNA in glucotoxic β-cells. We showed that dRib stimulation suppressed the expression of PDX-1 and insulin mRNA and lowered insulin protein levels, and that LA pretreatment completely reversed those changes (Fig. 3). These findings suggest that dRib might cause an oxidative stress-induced decrease in insulin gene expression in the same way as glucose. If this suggestion is correct, LA treatment might prevent glucose toxicity in pancreatic islets. It was reported that LA treatment attenuated oxidative damage in isolated islets, but, to our knowledge, no study on islets has assessed the effect of LA on glucose toxicity. Thus, we are planning a study to investigate whether LA can prevent glucose toxicity in rat islets.

There are some limitations in our study. We could not elucidate the mechanism for the increase in intracellular glutathione production induced by LA. We think that GCL or its substrates should be a target of future research. Furthermore, ROS and glutathione assays were not performed on isolated islets because of a shortage of tissues for experimentation. Therefore, there is no direct evidence that LA treatment preserves insulin expression through an antioxidant mechanism in isolated islets. However, we can hypothesize on the mechanism of action of LA in islets based on the cell line experiments.

LA treatment counteracted the oxidative damage and suppression of insulin expression caused by dRib stimulation of pancreatic β-cells. The protective mechanism of LA is believed to function via the augmentation of total intracellular glutathione biosynthesis rather than by the scavenging of ROS. Considering that glucose toxicity is mediated by oxidative stress, LA has the potential to become an agent for lessening this pathology. A future study is required for identifying the protective effect of LA against glucose toxicity.

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REFERENCES

11) Lee BW, Kwon SJ, Chae HY, Kang JG, Kim CS, Lee SJ, Yoo HJ,


