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2-Deoxy-D-ribose induces cellular damage by increasing oxidative stress and protein glycation in a pancreatic β-cell line

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Abstract

2-Deoxy-D-ribose (dRib) is a sugar with a high reducing capacity. We previously reported that dRib induced damage in pancreatic β-cells. The aim of this study was to investigate the mechanism of dRib-induced β-cell damage. 2-Deoxy-D-ribose provoked cytotoxicity and apoptosis within 24 hours in HIT-T15 cells. Three antiglycating agents—diethylenetriaminepentaacetic acid, aminoguanidine, and pyridoxamine—dose dependently inhibited dRib-triggered cytotoxicity and significantly suppressed apoptosis induced by dRib. 2-Deoxy-D-ribose increased intracellular reactive oxygen species and protein carbonyl levels in a dose-dependent manner. Diethylenetriaminepentaacetic acid and aminoguanidine significantly reduced dRib-induced rises in intracellular reactive oxygen species. All 3 inhibitors decreased the production of intracellular protein carbonyls by dRib. On incubation with albumin, dRib increased dicarbonyl and advanced glycation end product formation. Aminoguanidine and pyridoxamine significantly decreased the dicarbonyl and advanced glycation end product augmentations. These results suggest that both oxidative stress and protein glycation are important mechanisms of dRib-induced damage in a pancreatic β-cell line.
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1. Introduction

Glucose toxicity of pancreatic β-cells is defined as irreversible β-cell damage, including apoptosis, caused by chronic exposure to high glucose levels in type 2 diabetes mellitus [1]. The mechanism of glucose toxicity is via increased oxidative stress through multiple pathways, including oxidative phosphorylation, protein glycation, glucose autoxidation, and reactions involving methylglyoxal, protein kinase C, and hexosamine [2,3]. Protein glycation, a nonenzymatic reaction between glucose and the free amino groups of proteins, begins with the conversion of reversible Schiff base adducts to form more stable, covalently bound Amadori rearrangement products, which in turn undergo further transformation to a variety of advanced glycation end products (AGE). This is called the *Maillard reaction* [4]. Large amounts of reactive oxygen species (ROS) are produced at many steps of the parallel and sequential glycation cascade [5]. Various types of AGE are also formed by reactive dicarbonyls, such as methylglyoxal, 3-deoxyglucosone, and glyoxal, rather than by the Maillard reaction [6]. Aminoguanidine and pyridoxamine are the most well-known inhibitors of AGE formation [7]. Aminoguanidine is a prototype of a reactive carbonyl scavenging agent that inhibits the formation of AGE [8]. Pyridoxamine is a post-Amadori inhibitor and a scavenger of reactive carbonyl products of the Maillard reaction [9]. Diethylenetriaminepentaacetic acid (DTPA), a strong metal chelator, also suppresses the production of AGE with its inhibition of the autoxidation of glucose and Amadori compounds [10,11].

It is difficult for glucose to produce toxic effects on β-cells in a laboratory because glucose has the lowest reducing capacity among monosaccharides [12]. For instance, Poitout et al [13] reported that it took high glucose about 20 weeks to decrease insulin messenger RNA levels in the β-cell line. Therefore, a powerful reducing sugar is required for the

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study of glucose toxicity. D-Ribose and D-fructose, sugars with a large reducing capacity, have been known to suppress insulin gene transcription and provoke oxidative stress–induced apoptosis of β-cells in 3 days [14,15]. It has been shown that 2-deoxy-D-ribose (dRib) was a strong reducing sugar with a higher reactivity than D-ribose and D-fructose [16]. We previously reported that dRib induced oxidative stress and apoptosis in a β-cell line within 24 hours [17]. However, the mechanism was not investigated in our previous report.

The purpose of this study is to elucidate the mechanism of dRib-induced oxidative stress and apoptosis in pancreatic β-cells. We examined whether antiglycating agents suppress the dRib-induced oxidative damage of the pancreatic β-cell line and prevent the protein glycation with dRib in vitro.

2. Materials and methods

2.1. Materials

2-Deoxy-D-ribose, DTPA, aminoguanidine (AG), pyridoxamine (PM), glyoxal, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), dichlorodihydrofluorescein diacetate (H_2DCF-DA), dihydrorhodamine 123 (DHR123), dimethylsulfoxide, 2,4-dinitrophenylhydrazine (2,4-DNP), trifluoroacetic acid, Triton X-100, and sodium borate solution were purchased from Sigma-Aldrich (St Louis, MO). Streptomycin sulfate, HEPES, trichloroacetic acid (TCA), guanidine hydrochloride, potassium phosphate monobasic, and potassium phosphate dibasic were obtained from Amresco (Solon, OH). Hydrochloric acid (HCl), ethanol, and ethyl acetate were from Merck (Darmstadt, Germany). RPMI-1640, Dulbecco phosphate-buffered saline (DPBS), trypsin, penicillin, and streptomycin were from Gibco Invitrogen (Grand Island, NY). Fetal bovine serum (FBS) was from HyClone (Logan, UT). All culture dishes were from BD Falcon (Franklin Lakes, NJ).

2.2. Cell culture

Insulin-secreting HIT-T15 cells were provided by the Korean Cell Line Bank (Seoul, Korea). Cells (passage 70-80) were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 mU/mL penicillin, and 100 mg/mL streptomycin. The cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere and subcultured by trypsinization with 0.05% trypsin–0.02% EDTA in Ca^{2+}- and Mg^{2+}-free DPBS when they reached about 70% confluence. Two days after the subculture, quiescence was induced by incubating for 24 hours in RPMI-1640 medium containing 0.5% FBS. Thereafter, the culture medium was replaced by fresh RPMI-1640 containing 0.5% FBS; and various concentrations of dRib were added to the medium after pretreatment with AG, PM, or DTPA for 30 minutes. The cultures were incubated for 8 or 24 hours.

2.3. Assessment of cell viability

The MTT assay of cell viability was performed following a well-described procedure with minor modifications [18]. HIT-T15 cells were plated in 24-well cell culture plates at a density of 2×10^5 cells per well. The cells were incubated with 40 mmol/L dRib, with or without glycation inhibitors, for 24 hours. Afterward, 100 μL of MTT solution (5 mg/mL in PBS) was added to each well containing 1 mL of the medium. After 4 hours of incubation, the media were removed; and formazan crystals were solubilized with 300 μL of dimethylsulfoxide. Absorbance was measured with a microplate absorbance reader (Sunrise; Tecan, Untersbergstrasse, Grödig, Austria) at 540 nm using a 650-nm filter as the reference. Cells incubated with culture medium alone, representing 100% viability, were included as a control in all experiments to allow estimation of the viability percentage of the cell samples.

2.4. Flow cytometric analysis for apoptosis

By staining cells with a combination of annexin V and propidium iodide (PI), it is possible to detect nonapoptotic live cells (annexin V negative/PI negative), early apoptotic cells (annexin V positive/PI negative), and late apoptotic or necrotic cells (both positive) by flow cytometry [19]. HIT-T15 cells were stained with fluorescein isothiocyanate (FITC)–conjugated annexin V and PI according to the manufacturer's instructions (Vybrant Apoptosis Assay Kit 2; Molecular Probes, Eugene, OR). Briefly, HIT-T15 cells were cultured in 6-well plates at a density of 5×10^5 cells per well. The cells were stimulated with 50 mmol/L dRib and glycation inhibitors for 24 hours. After a gentle rinse with PBS, the cells were collected by trypsinization and centrifugation. Cell pellets were resuspended in PBS and centrifuged. The pellets were resuspended and incubated in the FITC-conjugated annexin V and PI staining solution at room temperature for 15 minutes. The stained cells were analyzed by a fluorescence-activated cell sorter (FACSCalibur; BD Bioscience, San Jose, CA) immediately, and quadrant data were calculated with CellQuest software (BD Bioscience). Ten thousand cells were evaluated for each sample.

2.5. Assessment of intracellular ROS levels

Intracellular oxidant levels were assessed using fluorescein-labeled dyes, H_2DCF-DA, and DHR123. Dichlorodihydrofluorescein diacetate is usually considered useful for reflecting the overall level of oxidative stress occurring in the cell cytoplasm, whereas DHR123 is particularly sensitive at detecting oxidants generated within mitochondria [20,21]. The cellular fluorescence intensity is directly proportional to the intracellular ROS levels. HIT-T15 cells were plated in 24-well cell culture plates at a density of 3×10^5 cells per well. The cells were stimulated with various concentrations of dRib and glycation inhibitors for only 8 hours because

extensive cell death could interfere with ROS measurements. Ten micromoles per liter H_2DCF-DA or 5 μmol/L DHR123 was added to cell cultures 30 minutes before harvesting. Cells were then washed with PBS twice and harvested using 0.05% trypsin. The cells were centrifuged and resuspended in PBS, and intracellular ROS levels were measured using a FACScan instrument (BD Bioscience). Twenty thousand cells were analyzed per sample. Results were calculated as the mean fluorescence intensity and expressed as the fold difference from control untreated cells.

2.6. Measurement of intracellular carbonyl contents

The protein carbonyl content was determined in cell lysates by the spectrophotometric assay of Levine et al [22]. In short, cells were cultured in 100-mm culture dishes at a density of 4×10^6 cells per well. The cells were incubated with various concentrations of dRib, with or without glycation inhibitors, for 24 hours. After 2 gentle rinses with PBS, the cells were collected by cell scrapers and centrifuged. Cell pellets were homogenized in 0.5-mL lysis buffer (1% Triton X-100 in DPBS) and incubated at room temperature for 10 to 15 minutes. The lysates were centrifuged (11 000*g* for 5 minutes) to remove debris. Afterward, 1 vol of streptomycin solution (10% streptomycin sulfate in 50 mmol/L HEPES) was added to 9 vol of supernatant and allowed to stand for 15 minutes. After centrifugation (11 000*g* for 5 minutes), the supernatant was used for determining the protein carbonyl content. The protein was precipitated with an equal volume of 20% TCA, kept on ice for 5 minutes, and then centrifuged (11 000*g* for 5 minutes). The precipitate was resuspended in 0.5 mL of 2,4-DNP solution (10 mmol/L 2,4-DNP in 2 N HCl), incubated in the dark at room temperature for 1 hour, and vortexed every 15 minutes. One milliliter of 20% TCA was again added to each tube, and the tubes were placed on ice and centrifuged (11 000*g* for 5 minutes). Afterward, pellets were washed 3 times in 1 mL of ethanol/ethyl acetate (1:1) to remove free agent, vortexed completely, and centrifuged (11 000*g* for 5 minutes). After centrifugation, the pellets were resuspended in 500 μL of guanidine solution (6 mol/L guanidine with 20 mmol/L potassium phosphate, adjusted to pH 2.3 with trifluoroacetic acid) by vortexing. The protein was fully redissolved by freezing overnight at −20°C and thawing, and insoluble materials were removed by additional centrifugation (11 000*g* for 5 minutes). The absorbance of the samples was measured at 366 nm using a spectrophotometer (UVmini-1240; Shimadzu, Nakagyo-ku, Kyoto, Japan) scanned against the blank that had been treated with only 2 N HCl, instead of the 2,4-DNP solution. The carbonyl content (in nanomoles per milliliter) was calculated from the peak absorbance (366 nm) using a molar absorption coefficient of 22 000 (mol/L)/cm. The amount of total cellular protein in the sample was determined using a BCA protein assay kit (Pierce, Rockford, IL). The intracellular carbonyl content was corrected for the total protein content (in micrograms per milliliter) and expressed as nanomoles per milligram protein.

2.7. Assessment of reactive dicarbonyl and AGE formation

The mixture contained 10 mg/mL of bovine serum albumin (BSA), 0.02% sodium azide, and 100 μmol/L $CuSO_4$ in 100 mmol/L sodium phosphate buffer (pH 7.4). The final volume was 4 mL. The mixture was incubated with various concentrations of dRib and glycation inhibitors at 37°C for 3 days in capped polystyrene tubes. All incubations were done in quadruplicate. The incubated sample mixtures were dialyzed against 100 mmol/L sodium phosphate buffer (pH 7.4) for 48 hours to remove extra, unbound dRib and any other impurities. They were stored frozen before assaying for dicarbonyl or AGE fluorescence.

Albumin-attached dicarbonyls were measured using the Girard-T reagent as described previously [23,24]. Briefly, the reaction mixture contained 60 μL of sample or each standard solution, 20 μL of 0.5 N sodium borate (pH 9.2), and 20 μL of 0.1 N Girard-T reagent and was incubated 30°C for 10 minutes. One hundred microliters of 0.1 N sodium borate (pH 9.2) was added, and the absorbance at 375 nm was measured against a zero blank using a spectrophotometer (Shimadzu). The concentration of reactive dicarbonyl was calculated by comparison with standard solutions and was corrected for the total protein concentration. We used glyoxal as a standard.

The AGE fluorescence of the incubation mixture was measured at the excitation and emission wavelengths of 350 and 450 nm, respectively, against an unincubated blank containing the protein, sugar, and inhibitor with a fluorescence reader (DTX 880 Multimode Detector; Beckman Coulter, Fullerton, CA). Any difference observed between the sample and the control was used as an indication of formation of AGE.

2.8. Statistical analysis

All data are given as means ± SE. The significance of data was determined by parametric analyses. The unpaired Student *t* test was used to compare 2 groups. Data from more than 2 groups were assessed by analysis of variance (ANOVA) followed by Duncan post hoc test. All analyses were performed using SPSS software (version 11.0; SPSS, Chicago, IL). Probability values (P) < 5% were considered significant.

3. Results

3.1. Glycation inhibitors suppress dRib-induced cytotoxicity

Diethylenetriaminepentaacetic acid, AG, and PM, which are inhibitors of protein glycation, were used in our study to investigate the mechanism of dRib-induced cell damage. HIT-T15 cells were incubated for 24 hours

in medium containing 40 mmol/L dRib after pretreatment with various concentrations of glycation inhibitors. An MTT assay showed that all glycation inhibitors significantly reduced dRib-mediated cytotoxicity in a dose-dependent manner. In detail, 40 mmol/L dRib stimulation markedly decreased the cell viability to about 16% of the control; but DTPA significantly prevented the dRib-mediated decrease in cell viability at concentrations of between 0.05 and 0.3 mmol/L (49%-68% cell viability) in a dose-dependent manner. Concentrations of 3 and 5 mmol/L AG (35% and 41% cell viability) and 0.5, 1, and 3 mmol/L PM (38%, 55%, and 67% cell viability) also dose dependently suppressed dRib-induced cytotoxicity (Fig. 1).

3.2. Glycation inhibitors reverse dRib-triggered apoptosis

A flow cytometric analysis with annexin V and PI double staining was performed to confirm whether dRib induces apoptosis and antiglycating agents suppress apoptosis in HIT-T15 cells. The analysis showed that 50 mmol/L dRib stimulation markedly decreased the percentage of viable cells and increased the percentage of apoptotic (annexin V positive) cells. However, pretreatment with 0.3 mmol/L DTPA, 5 mmol/L AG, or 3 mmol/L PM significantly reversed the dRib-triggered apoptosis (Table 1 and Supplementary Fig. 1).

3.3. dRib stimulates intracellular ROS production

To evaluate the effect of dRib on ROS levels in HIT-T15 cells, we performed flow cytometric analyses using H_2DCF-DA (Fig. 2A) and DHR123 (Fig. 2B) dyes. Various concentrations of dRib dose dependently increased the fluorescence of H_2DCF-DA and DHR123 in HIT-T15 cells. Stimulation with 10 and 30 mmol/L dRib showed increasing tendencies in fluorescence with both dyes compared with the control. At 50 and 70 mmol/L dRib, there was a marked increase in the fluorescence, particularly with the DHR123 dye. Pretreatments of HIT-T15 cells with 0.3 mmol/L DTPA or 5 mmol/L AG significantly inhibited the dRib-induced rises in H_2DCF-DA and DHR123 fluorescence levels. Three millimoles per liter PM, however, did not decrease the dRib-induced fluorescence (Table 2 and Supplementary Fig. 2).

Table 1
Percentage of annexin V–positive cells in control and dRib-treated HIT-T15 cells with and without glycation inhibitors

	Annexin V–positive cells (%)
Control	7.9 ± 1.5
50 mmol/L dRib alone	83.5 ± 9.3*
50 mmol/L dRib + 0.3 mmol/L DTPA	29.4 ± 0.9†
50 mmol/L dRib + 5 mmol/L AG	31.3 ± 6.8†
50 mmol/L dRib + 3 mmol/L PM	18.8 ± 1.0†

Data are expressed as means ± SE of 4 independent experiments.
* $P < .01$ vs control by 1-way ANOVA with Duncan post hoc test.
† $P < .01$ vs 50 mmol/L dRib alone by 1-way ANOVA with Duncan post hoc test.

3.4. dRib increases intracellular carbonyl contents

An increased level of carbonyl groups is an indicator of the oxidative modification of amino acids, including the glycation reaction [25]. In comparison with 0, 10 and 20 mmol/L dRib showed an increase and 30 and 40 mmol/L dRib dose-dependently increased the level of intracellular protein carbonyls (Fig. 3A). Not only 0.3 mmol/L DTPA and 5 mmol/L AG but also 3 mmol/L PM significantly suppressed the dRib-stimulated elevation in intracellular carbonyl levels (Fig. 3B).

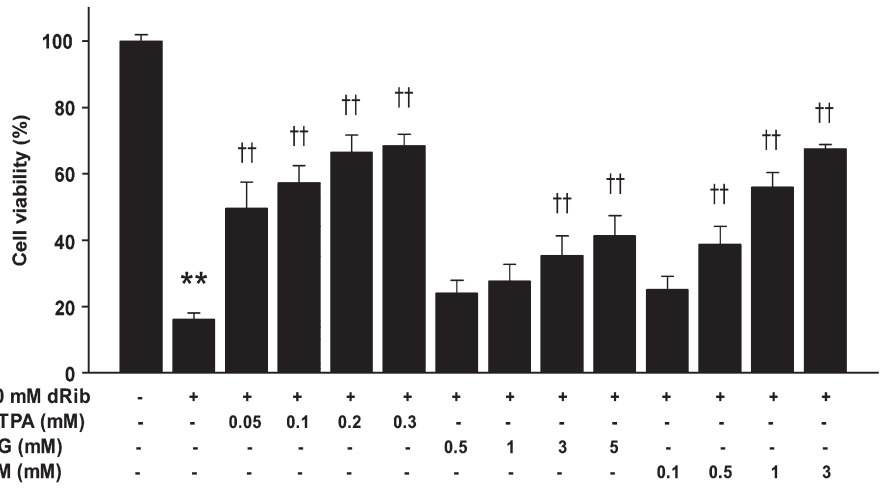

Fig. 1. Protective effects of DTPA, AG, and PM on dRib-triggered cytotoxicity. HIT-T15 cells were preincubated with DTPA, AG, and PM for 30 minutes at the indicated concentrations and then cultured with 40 mmol/L dRib for 24 hours. Cell viability was determined by an MTT assay. Data are expressed as the mean ± SE of the percentage of viable cells relative to the untreated control. This experiment was performed twice, in quadruplicate. **$P < .01$ vs control; ††$P < .01$ vs 40 mmol/L dRib alone by 1-way ANOVA with Duncan post hoc test.

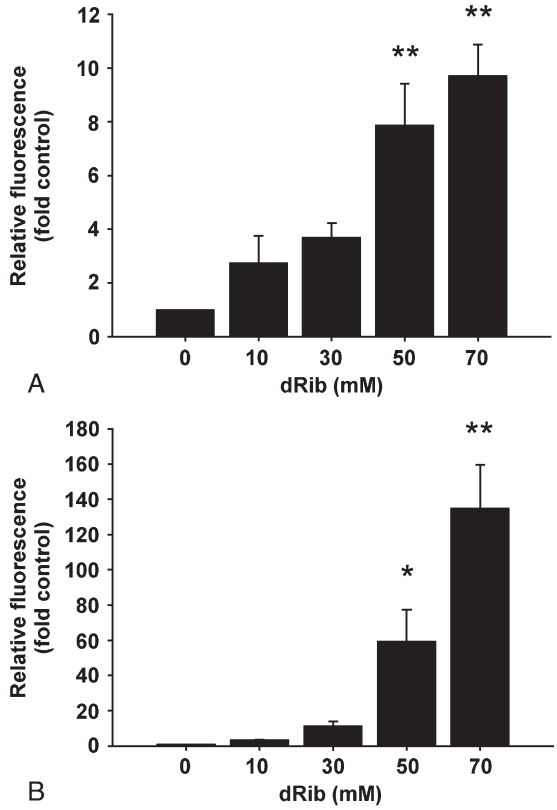

Fig. 2. Dose responses of dRib to intracellular ROS levels. The production of ROS was studied by flow cytometry using the fluorescent dyes H_2DCF-DA (A) and DHR123 (B). HIT-T15 cells were cultured with the indicated concentrations of dRib for 8 hours. Cells were incubated with 10 μmol/L H_2DCF-DA (A) and 5 μmol/L DHR123 (B) during the final 30 minutes. Results were calculated as the fold difference from untreated control cells. Data are expressed as means ± SE of 4 independent experiments. *$P < .05$ and **$P < .01$ vs control by 1-way ANOVA with Duncan post hoc test.

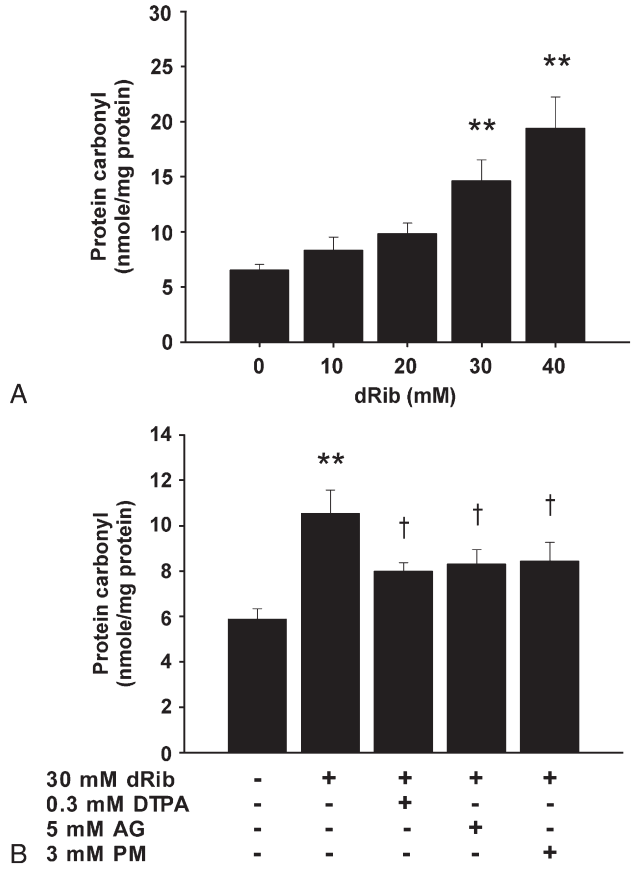

Fig. 3. Protein carbonyl assays in HIT-T15 cells treated with dRib and glycation inhibitors. HIT-T15 cells were cultured with the indicated concentrations of dRib for 24 hours (A). The cells were preincubated with 0.3 mmol/L DTPA, 5 mmol/L AG, or 3 mmol/L PM for 30 minutes and then cultured with 30 mmol/L dRib for 24 hours (B). The intracellular carbonyl content was corrected for the total protein content and expressed as nanomoles per milligram protein. Each bar represents the means ± SE. The carbonyl measurement was performed twice, in quadruplicate. **$P < .01$ vs control and †$P < .05$ vs 30 mmol/L dRib alone by 1-way ANOVA with Duncan post hoc test.

3.5. dRib increases protein-bound dicarbonyl and AGE formations

Dicarbonyls formed during monosaccharide autoxidation are reactive intermediates in the formation of AGE [6,10]. To demonstrate dRib-induced protein glycation, we measured the protein-bound dicarbonyls and the fluorescence of AGE through in vitro BSA glycation with dRib.

Figs. 4 and 5 show the dicarbonyl concentration and AGE fluorescence according to the concentration of dRib in the incubation mixture containing BSA. As shown, there were linear increases in the dicarbonyl amount and AGE fluorescence as the concentration of dRib increased. These increases were not suppressed by the addition of 0.3 mmol/L DTPA. However, the pretreatment with 5 mmol/L AG significantly attenuated the dicarbonyl and fluorescence augmentations at 30 and 50 mmol/L dRib, but not at 10 mmol/L dRib. The addition of 3 mmol/L PM exhibited inhibitory effects on dicarbonyl formation at 50 mmol/L dRib and fluorescence increases at 30 and 50 mmol/L dRib (Figs. 4 and 5).

Table 2
Relative intracellular ROS levels in control and dRib-treated HIT-T15 cells with and without glycation inhibitors

	Relative fluorescence (fold control)	
	H_2DCF-DA	DHR123
Control	1.0	1.0
70 mmol/L dRib alone	10.7 ± 1.5*	103.1 ± 19.8*
70 mmol/L dRib + 0.3 mmol/L DTPA	6.2 ± 0.3†	31.0 ± 7.3†
70 mmol/L dRib + 5 mmol/L AG	6.7 ± 0.5†	47.5 ± 6.0†
70 mmol/L dRib + 3 mmol/L PM	10.0 ± 0.5	97.6 ± 18.5

Data are expressed as means ± SE of 4 independent experiments.
* $P < .01$ vs control by 1-way ANOVA with Duncan post hoc test.
† $P < .05$ vs 70 mmol/L dRib alone by 1-way ANOVA with Duncan post hoc test.

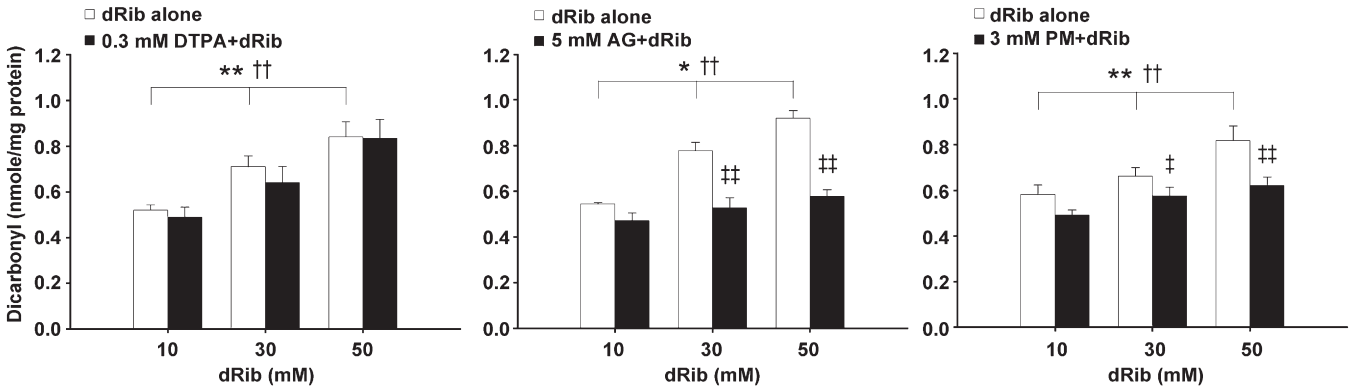

Fig. 4. Inhibitory effects of DTPA, AG, and PM on protein-bound dicarbonyl formation. Bovine serum albumin (10 mg/mL) was incubated with 10, 30, and 50 mmol/L dRib for 3 days at 37°C in the presence and absence of DTPA, AG, and PM. Dicarbonyl concentrations were estimated by adduct formation with the Girard-T reagent. Each bar represents the means ± SE. This experiment was performed twice, in quadruplicate. $^{*}P < .05$ and $^{**}P < .01$ among 10, 30, and 50 mmol/L dRib alone by 1-way ANOVA. $^{\dagger\dagger}P < .01$ across the groups (the linear trend test). $^{\ddagger}P < .05$ and $^{\ddagger\ddagger}P < .01$ vs 10, 30, or 50 mmol/L dRib alone by Student *t* test.

4. Discussion

Many researchers have tried to study glucose toxicity to investigate the mechanism of β-cell failure in a laboratory. However, glucose has a very low reducing capacity and requires a long time to provoke oxidative stress, although it is a physiologic property [13,26]. Hydrogen peroxide [27,28], reducing sugars [15,29], and other oxidative stress–inducing agents have been used in the study of glucose toxicity in vitro. Hydrogen peroxide is a direct oxidizing agent and quickly disrupts cell structure. Some reducing sugars have been shown to have high reactivities, and they may go through a process similar to glucose [12,30]. Therefore, we chose dRib as a stimulating agent because it is known as a sugar to have strong reducing power [16].

In this report, we showed that dRib dose dependently increased intracellular ROS levels in HIT-T15 cells. The ROS may have originated from the mitochondria because DHR123 fluorescence was much stronger than that with H_2DCF-DA, and the dRib induced cytotoxicity and apoptosis in a dose-dependent manner. We previously reported that *N*-acetyl-L-cysteine, a hydrogen peroxide–eliminating agent, reversed dRib-induced oxidative stress and apoptosis in HIT-T15 cells [17]. Accordingly, there is no doubt that dRib-induced cytotoxicity and apoptosis are mediated by oxidative stress. The dRib must operate via the same mechanism as glucose because it is accepted as a glucotoxicity-inducing agent. Thus, we intended to investigate protein glycation, one of mechanisms of glucose toxicity. We chose in vitro glycation with BSA because there were no simple methods for the measurement of intracellular intermediates and no specific inhibitors during the glycation processes [31]. Our results indicated that dRib dose dependently increased AGE and its precursor dicarbonyl.

This study showed that DTPA, AG, and PM, well-known inhibitors of protein glycation, partly but significantly

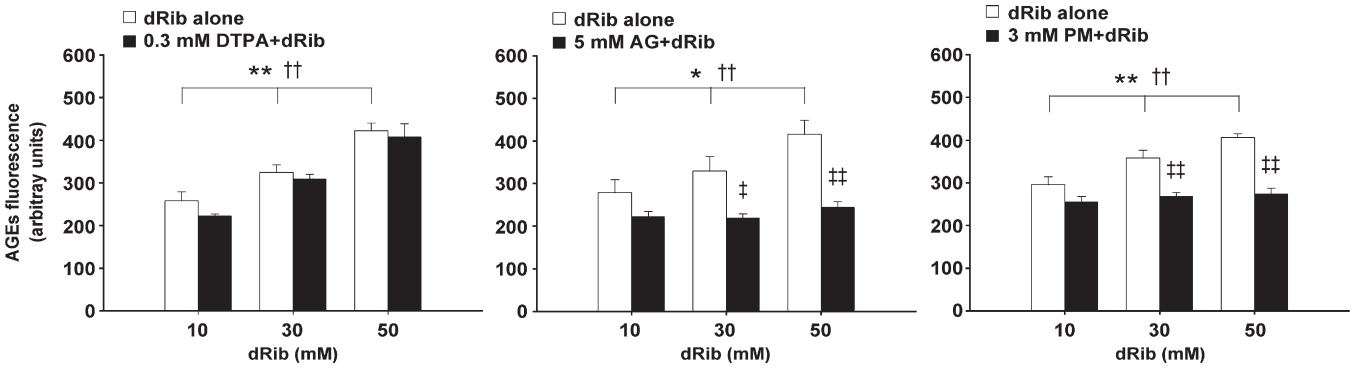

Fig. 5. Inhibitory effects of DTPA, AG, and PM on formation of AGE. Bovine serum albumin (10 mg/mL) was incubated with 10, 30, and 50 mmol/L dRib for 3 days at 37°C in the presence and absence of DTPA, AG, and PM. Advanced albumin glycation was determined by measurement of fluorescence intensity (excitation and emission wavelength of 350 and 450 nm, respectively) vs unincubated blank containing the protein, dRib, and inhibitor. Each bar represents the mean ± SE. This experiment was performed twice, in quadruplicate. $^{*}P < .05$ and $^{**}P < .01$ among 10, 30, and 50 mmol/L dRib alone by 1-way ANOVA. $^{\dagger\dagger}P < .01$ across the groups (the linear trend test). $^{\ddagger}P < .05$ and $^{\ddagger\ddagger}P < .01$ vs 10, 30, or 50 mmol/L dRib by Student *t* test.

suppressed dRib-induced cytotoxicity and apoptosis. This result suggests that dRib induces cell damage through protein glycation. In detail, DTPA attenuated the dRib-induced increase in intracellular ROS levels, but did not inhibit dicarbonyl and AGE formations in vitro. This means that DTPA inhibited dRib-induced oxidative stress but could not suppress the dRib-mediated protein glycation. Diethylenetriaminepentaacetic acid might show a ROS-suppressive effect by inhibiting a superoxide-driven Fenton reaction to generate hydroxyl radicals because it is a strong metal chelator [32]. In contrast, PM did not suppress dRib-induced ROS production, but did inhibit both dicarbonyl and AGE formations triggered by dRib. Pyridoxamine might inhibit AGE formation through trapping dicarbonyl intermediates in vitro. It has been proposed that PM scavenges dicarbonyls produced during glycation in addition to its inhibition of post-Amadori protein modifications [24]. Conflicting results between DTPA and PM suggest that 2 independent mechanisms, oxidative stress and protein glycation, mediate the dRib-induced cytotoxicity and apoptosis. Oxidative stress is not necessarily implicated in protein glycation. Litchfield et al [33] reported that the glycation reaction could proceed even in the absence of oxygen and in the presence of metal chelators. In our work, dRib presumably mediated protein glycation via a nonoxidative mechanism. Hence, DTPA could not prevent dRib-mediated dicarbonyl and AGE formations; but PM could inhibit dRib-induced cytotoxicity and apoptosis despite its low antioxidative effect. Both DTPA and PM, however, significantly suppressed intracellular protein carbonyls produced by dRib. Protein carbonyls are considered a broad marker of oxidative damage, and their elevation is generally a sign not only of oxidative stress but also of glycation and glycoxidation [25]. Therefore, the protein carbonyl assay seems to be appropriate for general assessment of dRib-induced damage. Our results also suggested that AG had antioxidative and antiglycating effects because AG decreased the dRib-induced rises in intracellular ROS and protein carbonyls, as well as dicarbonyl and AGE formations in vitro. This suggestion is supported by a previous report that AG could quench hydroxyl radicals, irrespective of its effects on AGE formation [34]. Based on our data from cellular experiments and in vitro glycation, we suppose that the dRib-triggered damage is mediated by both oxidative stress and protein glycation in HIT-T15 cells.

Protein glycation, a nonenzymatic posttranslational protein modification between reducing sugars and protein, has been implicated in the pathogenesis of various disorders, including chronic diabetic complications, Alzheimer disease [35], uremia [36], atherosclerosis [37], and physiologic aging [38]. There are multiple pathways of AGE formation and multiple sites of ROS production during the glycation process [31,39]. It used to be thought that AGE could only be formed by the Maillard reaction. However, it has been found in recent years that glucose-derived dicarbonyls are more effective precursors of AGE [6]. The reactive dicarbonyls arise from autoxidative reactions of free sugars (Wolff pathway) [10,40], Schiff base adducts (Namiki pathway) [41], and Amadori intermediates [42,43]. Our research demonstrated that dRib produced both AGE and dicarbonyls during incubation with albumin. This result means that dRib may induce AGE formation via a non-Maillard pathway in vitro. Our study also showed that dRib caused cellular damage within 24 hours in HIT-T15 cells, but it took 3 days to produce dicarbonyls and AGE in vitro glycation with albumin. These results suggest that early glycation processes, such as monosaccharide autoxidation, Schiff reaction, etc, are possible mechanisms of dRib-induced damage in HIT-T15 cells.

In summary, this study has shown that dRib increased intracellular ROS and protein carbonyl levels, and cytotoxicity and apoptosis in HIT-T15 cells, and augmented dicarbonyl and AGE formation by in vitro glycation with albumin. In addition, common glycation inhibitors suppressed dRib-induced oxidative damage and apoptosis. Taken together, dRib promptly provokes cytotoxicity and apoptosis in a pancreatic β-cell line; and both oxidative stress and protein glycation are important mechanisms of β-cell damage. Hence, dRib can be used for the study of glucose toxicity or oxidative stress in β-cells. Furthermore, it can be applied to the experimental study of other diabetic complications and neurodegenerative disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.07.028.

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