Hypoglycemic effect of resveratrol in type 2 diabetic model db/db mice and its actions in cultured L6 myotubes and RIN-5F pancreatic β-cells

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Resveratrol, a phytoalexin present in the skin of grapes and red wine, has been demonstrated to possess a wide range of health-promoting activities including anti-diabetic properties. In the present study, we investigated the effect of resveratrol in both type 2 diabetic mice and cell culture systems. In cultured L6 myotubes, we studied the effect of resveratrol on glucose uptake and translocation of glucose transporter 4 to plasma membrane from the aspects of insulin signaling and AMP-activated protein kinase signaling. In cultured RIN-5F cells, we examined whether resveratrol would protect the pancreas-derived β-cells from oxidative stress. Resveratrol significantly suppressed the elevation in the fasting blood glucose level and the serum triglyceride and lipid peroxide levels in db/db mice. Resveratrol stimulated glucose uptake and glucose transporter 4 translocation by activating both insulin signaling and AMP-activated protein kinase signaling. Moreover, resveratrol could protect pancreatic β-cells from advanced glycation end products-induced oxidative stress and apoptosis. From these results, resveratrol is suggested to show anti-diabetic effect by stimulating both insulin-dependent and -independent glucose uptake in muscles and by protecting pancreatic β-cells from advanced glycation end products-induced oxidative stress and apoptosis.

Key Words: AMPK signaling, myotube, pancreatic β-cell, resveratrol, type 2 diabetic db/db mouse

The total number of people with diabetes is increasing worldwide. To reduce the hyperglycemia, several trials have been conducted; for instance, inhibition of α-glucosidase to interfere with glucose absorption from the intestine, stimulation of pancreatic islet cells to secret insulin, reduction of hepatic glucose production, and augmentation of glucose utilization.1,2 Type 2 (non-insulin-dependent) diabetes is associated with metabolic syndrome and a marked increase in the risk of cardiovascular morbidity and mortality.3–7 Under diabetic condition, chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) increase insulin resistance and deteriorate β-cell functions which lead to the aggravation of type 2 diabetes.8 The skeletal muscles which account for the majority (∼80%) of insulin-mediated glucose uptake in the post-prandial state play an important role in maintaining glucose homeostasis.9 In skeletal muscles, insulin increases glucose uptake via a signaling that leads to activation of phosphatidylinositol-3 kinase (PI3K) and Akt, resulting in increased translocation of glucose transporter 4 (GLUT4) to the plasma membrane.10 Therefore, skeletal muscle tissue implies an attractive therapeutic target for the treatment of insulin resistance and type 2 diabetes. There is another GLUT4 translocation promoter, the AMP-activated protein kinase (AMPK) which is composed of three subunits, α, β and γ.10 In mammalian cells, AMPK activated by an increase in AMP/ATP ratio acts as an energy sensor,10 and has at least two upstream kinases, LKB1 and calmodulin-dependent protein kinase kinase (CaMKK).10 AMPK is activated by exercise/contraction11 and numerous compounds including metformin12 and thiazolidinedione,13 resulting in stimulation of glucose uptake in skeletal muscles. The study of novel compounds that activate AMPK and increase skeletal muscle glucose uptake could be used for the development of new treatment of insulin resistance and type 2 diabetes. In the hyperglycemic state, glucose reacts nonenzymatically with protein amino groups to initiate post-translational modification process known as nonenzymatic glycosylation, or glycation. This process begins with the conversion of reversible Schiff base adducts to more stable, covalently bound Amadori rearrangement products.13 Over the course of weeks to months, the Amadori products undergo further rearrangement reactions to form irreversibly bound moieties called advanced glycation end products (AGEs).14,15 Increased oxidative stress and AGEs may result in the overexpression of receptor of AGEs (RAGE),16,17 which is minimally expressed in normal vasculature.18 The RAGE gene promoter contains NF-κB binding sites, which positively control RAGE expression and link RAGE to the inflammatory response.19 Up-regulation of RAGE has been shown to occur in endothelial cells, smooth muscle cells, and mononuclear phagocytes in the diabetic vasculature.20,21 The binding of AGEs to RAGE activates multiple signaling cascades, including extra-cellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK), and the generation of ROS,22 ROS augmentation reportedly deteriorates β-cell functions, because gene expression of antioxidant enzymes such as glutathione peroxidase and catalase was very low in pancreatic cells.23,24 Thus, β-cells are vulnerable to oxidative stress, leading to the reduction of insulin secretion.

Resveratrol (3,5,4′-trans-trihydroxystilbene), a phytoalexin polyphenol that is found naturally in such as grapes, peanuts and mulberries. Resveratrol has a number of health benefits including anticarcinogenic,25 cardiovascular protective26 and estrogenic activities27 as well as anti-inflammatory,28 free-radical scavenging29 activities, inhibition/induction of apoptosis,30 inhibition of platelet aggregation,31 life-extension,32 and neuroprotection.33

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We have reported that resveratrol shows anti-invasive effect against hepatoma cells, and hypolipidemic effect in glomerulonephritic and hepatoma-bearing rats.

Although a number of studies concerning the effect of resveratrol on diabetes or hyperglycemia have been reported, little has been validated in type 2 diabetes model animals. Hence, the present study is intended to investigate comprehensive effect of resveratrol on metabolic functions in type 2 diabetic model animals (db/db mice) as well as myotubes and pancreatic β-cells in culture. The db/db mice are characterized by obesity, insulin resistance, severe hyperglycemia, pancreatic injury and cardiovascular complications, and therefore regarded as a useful model of heavy human type 2 diabetes.

Materials and Methods

Materials. L6 myoblasts derived from a rat and RIN-5F cells derived from rat pancreatic β-cells were purchased from American Type Culture Collection (Manassas, VA; ATCC® numbers: CRL-1458 and CRL-2058, respectively), Dulbecco’s modified Eagle medium (DMEM) and RPMI 1640 medium were from Biological Fluids, Inc. (Rockville, MD), fetal bovine serum (FBS) was from JRH Biosciences, (Lenexa, KS). Resveratrol, bovine serum albumin (BSA, fatty acid free) and Triton X-100 were purchased from Sigma Chemical Co., (St. Louis, MO). Glucose, total cholesterol (T-Ch) and triglyceride (TG) assay kits (Glucose CII Test Wako, Cholesterol E-Test Wako and Triglyceride E-Test Wako, respectively) were from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). Serum lipid peroxide was estimated as thiobarbituric acid-reactive substances (TBARS) with a commercial kit (TBARS Assay Kit, ZeptoMetrix Corporation, Buffalo, NY). Insulin assay kit was from Morris Institute of Biological Science, Inc., (Yokohama, Japan). The anti-phospho-Akt (Ser473) antibody was obtained from Upstate Biotechnology (Lake Placid, NY). The anti-Akt1 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-phospho-AMPKα (Thr172) and anti-AMPKα antibodies were obtained from Cell Signaling Technology, Inc., (Beverly, MA). Anti-Na+/K+ ATPase α-1 antibody from Millipore (Billerica, MA), anti-GLUT4 antibody from Abd Serotec (Oxford, UK), horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG antibodies from Invitrogen (San Diego, CA). All other chemicals were of the best grade commercially available, unless otherwise noted. Plastic multiwell plates and tubes were obtained from Nunc A/S (Roskilde, Denmark) or Iwaki brand (Asahi Glass Co., Ltd., Tokyo, Japan).

Effect of resveratrol on blood glucose levels in db/db mice. All animal experiments were conducted in accordance with guidelines established by the Animal Care and Use Committee of Tokyo University of Agriculture and Technology and were approved by this committee. To determine the effect of resveratrol on fasting blood glucose levels, db/db mice were used as a model of type 2 diabetes. Male db/db and its misty (m/m) control (normal) mice (5 weeks of age) were obtained from Charles River Japan, Kanagawa, Japan. Animals were individually housed in stainless-steel cages with wire bottoms in an air-conditioned room with a temperature of 22 ± 2°C, a relative humidity of 60 ± 5%, and an 8:00–20:00 light cycle. All mice were maintained on a stock CE-2 pellet diet (CLEA Japan, Tokyo, Japan) for 3 days and thereafter a basal 20% casein diet (20C) for 4 days. The composition of the 20C diet was as follows (dry weight basis): 20% casein (Oriental Yeast Co., Tokyo, Japan), 7% corn oil (Hayashi Chemicals Co., Tokyo, Japan), 13.2% α-cornstarch (Nihon Nakanos Kogyo Co., Yokohama, Japan), 49.75% β-cornstarch (Nihon Nakanos Kogyo Co.), 3.5% mineral mixture (AIN-93G composition, Nihon Nakanos Kogyo Co.), 1% vitamin mixture (AIN-93 composition, Nihon Nakanos Kogyo Co.), 0.25% choline bitartrate (Wako Pure Chemical Industries), 0.3% L-cystine (Wako Pure Chemical Industries), and 5% cellulose powder (Oriental Yeast Co.). After preliminary feeding for 1 week, mice were deprived of their diet at 9:00 a.m. and allowed free access to water until blood collection from tail vein 4 h later. Blood (10 μl) was burst in water (40 μl), 20% (w/v) trichloroacetic acid aqueous solution (50 μl) and test tubes containing the mixture were kept in ice-cold water. The mixture was then centrifuged at 13,000 × g and 4°C for 5 min. The resultant supernatant (80 μl) was subjected to glucose determination with a commercial kit and a spectrophotometer (Model U-1100, Hitachi Science Systems, Ltd., Ibaraki, Japan) at 505 nm, and db/db mice (6 weeks of age at the moment) were divided into two groups of similar fasting blood glucose levels and body weights (0 week). Diabetic mice of each of the two groups were given either the 20C as the control group or the 20C supplemented with 0.04% resveratrol as the test group for 5 weeks. Resveratrol was supplemented to the 20C at the expense of β-cornstarch. Likewise, misty control mice were given the 20C as the normal group for 5 weeks.

Syntheses of advanced glycation end products (AGEs). AGEs were generated from co-incubation of BSA with either D-glucose (AGE1) or D-glyceraldehyde (AGE2) according to the method of Kume et al. AGE1 and AGE2 were incubated at 37°C for 8 weeks and 2 weeks, respectively. BSA alone was incubated at 37°C for 2 weeks under conditions without any carbohydrates, and employed as the control for AGE1 and AGE2. This BSA was designated as CNT.

Determination of glucose uptake by cultured L6 myotubes. Stock cultures of L6 myoblasts were maintained in DMEM supplemented with 10% (v/v) FBS, streptomycin (100 μg/ml), and penicillin G (100 U/ml) (10% FBS/DMEM) under an atmosphere of 5% CO2/95% humidified air at 37°C as described previously. Effect of resveratrol was examined by the procedure described previously with slight modifications. Briefly, the L6 myoblasts (5 × 10³ cells/well) were subcultured into Nunc 24-place multiwell plates and grown for 11 days to form myotubes in 0.4 ml of 10% FBS/DMEM. The medium was renewed every 3 days. Later, the 11-day-old myotubes were kept for 2 h in filter-sterilized Krebs-Henseleit buffer (pH 7.4, 141 mg/l MgSO₄, 370 mg/l KH₂PO₄, 350 mg/l KCl, 6900 mg/l NaCl, 373 mg/l CaCl₂-2H₂O and 2100 mg/l NaHCO₃) containing 0.1% bovine serum albumin, 10 mM Heps and 2 mM sodium pyruvate (KHH buffer). The myotubes were thereafter cultured in KHH buffer containing 11 mM glucose without or with resveratrol (1–100 μM) for another 4 h. Glucose concentrations in KHH buffer were determined with a glucose assay kit and a microplate reader (Applied, Thermo Fisher Scientific Inc., MA) at 508 nm, and the amounts of glucose consumed were calculated from the differences in glucose concentrations before and after culture.

Preparation of plasma membrane from L6 myotubes. The L6 myoblasts (5 × 10⁵ cells) were subcultured into Nunc 60 mm dishes and grown for 11 days to form myotubes in 3 ml of 10% FBS/DMEM. The medium was renewed every 3 days. Later, the 11-day-old myotubes were kept for 2 h in KHH buffer. Then, the myotubes were cultured in KHH buffer containing 11 mM glucose without or with resveratrol (10, 100 μM) for appropriate time intervals. Then, plasma membrane fractions were obtained by the method described by Nishiumi and Ashida. Briefly, to prepare the plasma membrane and post-plasma membrane fraction, L6 myotubes were harvested with buffer A (50 mM Tris-HCl (pH 8.0), 0.5 mM DTT, protease inhibitor cocktail and 1 mM NaVO₃) (Nacalai Tesque, Inc., Kyoto, Japan) containing 0.1% (v/v) IGEPAL CA-630 (Sigma Chemical Co.), and homogenized with 21-gauge needle. Each homogenate was centrifuged at 1,000 × g for 20 min at 4°C, and the precipitate was suspended in IGEPAL CA-630-free buffer A, and recentrifuged at 1,000 × g for 10 min at 4°C. The precipitate obtained was suspended again.
in buffer A containing 1.0% (v/v) IGEPAL CA-630, stood on ice for 1 h with occasional mixing and centrifuged at 16,000 × g for 20 min at 4°C. The supernatant was collected and stored as the plasma membrane fraction at −80°C until analyses. The supernatant from the first 1,000 × g centrifugation was gathered and centrifuged again at 16,000 × g for 20 min at 4°C. The supernatant obtained was collected and used as a post-plasma membrane fraction.

**Preparation of cell lysate.** The 11-day-old myotubes were kept for 2 h in KHH buffer. Then, the myotubes were cultured in KHH buffer containing 11 mM glucose with resveratrol (10 μM) for appropriate time intervals. The cells were scraped from the plates into ice-cold RIPA lysis buffer (Nacalai Tesque) and centrifuged at 20 min at 4°C and 16,000 × g. The supernatant was collected and stored at −80°C until use.

**Western blotting.** Protein samples were separated by SDS–PAGE, transferred to a PVDF membrane which was blocked for 1 h with 5% (w/v) dry milk in Tris-buffered saline, and incubated overnight at 4°C with the primary antibody. The primary antibody was detected with IRP-conjugated anti-rabbit or anti-mouse secondary antibody and visualized by using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fischer, Rockford, IL).

**Measurement of intracellular ROS.** RIN-5F cells derived from rat pancreatic β-cells were maintained in RPMI 1640 supplemented with 10% (v/v) FBS, streptomycin (100 μg/ml), and penicillin G (100 U/ml) (10% FBS/RPMI 1640) under an atmosphere of 5% CO2/95% humidified air at 37°C. The medium was renewed every 3 days. The cells (5 × 105 cells/well) were cultured into Nunc 12-place multiwell plates. After being cultured for 72 h in 1 ml of 10% FBS/RPMI 1640, the medium in each well was removed. Thereafter, RIN-5F cells received 1 ml of fresh medium (1% FBS/RPMI 1640) without or with resveratrol and AGEs for another 3 h. Effect of resveratrol on oxidative stress was examined by measurement of intracellular ROS based on ROS-mediated conversion of non-fluorescent 2',7'-DCFH-DA into DCFH. The intensity of fluorescence reflects enhanced oxidative stress. After the 3 h incubation, RIN-5F cells were incubated with DCFH-DA (final 25 μM) in 1% FBS/RPMI 1640 at 37°C for 20 min. At the end of the incubation, DCFH fluorescence of the cells from each well was measured at an emission wavelength of 530 nm and an excitation wavelength of 488 nm using a flow cytometer (Becton Dickinson, San Jose, CA).

**Flow cytometric evaluation of apoptosis.** RIN-5F cells received 1 ml of fresh medium (1% FBS/RPMI 1640) containing 200 μg BSA alone (CNT) or AGE2 without or with 50 μM resveratrol for another 6 h. Effect of resveratrol on oxidative stress-induced apoptosis was examined by using an Annexin V-FITC apoptosis detection kit (Becton Dickinson, San Jose, CA). Annexin V has a strong, Ca2+-dependent affinity for phosphatidylserine (PS), which translocates from the internal to the external surface of the plasma membrane as a probe for detecting apoptosis. Samples were incubated on ice for 10 min in the dark with Annexin V and propidium iodide (PI) and analyzed with a flow cytometer.

**Statistical analysis.** Data are expressed as means ± standard errors of means (SEM). Multigroup comparisons were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test (Instat ver 2.00, GraphPad Software Inc., San Diego, CA). Values of p < 0.05 were considered statistically significant.

**Results**

**Effect of resveratrol on blood glucose level and other parameters in db/db mice.** To study the in vivo effect of resveratrol, we employed db/db mice, a model for type 2 diabetes. Fig. 1A shows the effect of resveratrol on the fasting blood glucose level. The blood glucose level linearly increased to the third week of feeding and thereafter maintained the high value up to fifth week in diabetic control mice, while it was unchanged and constant in normal mice for this period. Resveratrol (0.04% in diet) tended to suppress until the second week of feeding and significantly suppressed the rise in the blood glucose level at the third week and thereafter. As shown in Fig. 1B, food intake of the resveratrol group for 5 weeks was almost the same as that of the control group, suggesting that the suppressive effect of resveratrol on fasting blood glucose level was not due to reduced food intake but due to its pharmacological action. As seen in Fig. 1C, T-Ch, TG and TBARS concentrations in serum of the diabetic control group significantly increased as compared with those of the normal group. Resveratrol, however, significantly suppressed the rises in the TG and TBARS concentrations. Serum insulin concentration in the control group tended to increase as compared with that in the normal group. The concentration in the resveratrol group also tended to increase as compared with that in the control group. However, these differences in insulin concentrations between the normal and diabetic control groups and between the diabetic control and resveratrol groups were not statistically significant (Fig. 1C).

**Resveratrol accelerated glucose uptake ability and effectively translocated GLUT4 to plasma membrane.** To investigate mechanisms for resveratrol actions in vivo, we first examined its effect on glucose uptake by L6 myotubes in vitro (Fig. 2). Resveratrol dose-dependently increased glucose uptake at concentrations of 1–100 μM in the absence of insulin.

Myotubes take glucose by mainly using GLUT4. Its recruitment to the surface of muscle cells is induced by both insulin and exercise.(33,45) Thus, we examined whether or not resveratrol would promote GLUT4 translocation to plasma membrane by western blotting analyses using antibodies to GLUT4 and Na+/K+ ATPase, the latter being a plasma membrane marker enzyme. Treatment of L6 myotubes with 100 μM resveratrol for 15 min promoted GLUT4 translocation (Fig. 3A). Phosphorylation ratios of AMPK and Akt commenced to increase 15 min after resveratrol (10 μM) treatment and thereafter further increased up to 240 min (Fig. 3 B and C). Time-dependent changes of GLUT4 translocation after resveratrol treatment are shown in Fig. 3 D and E. While maximum translocation of GLUT4 was attained 5–10 min after treatment and thereafter GLUT4 gradually diminished at the low concentration of resveratrol (10 μM), GLUT4 rapidly translocated to plasma membrane 2.5 min after treatment and maintained the promotive effect up to 40 min at the high concentration of resveratrol (100 μM).

**Resveratrol protects pancreatic β-cells from oxidative stress.** As above-mentioned, β-cells are vulnerable to oxidative stress. Resveratrol is one of polyphenols with antioxidative property. Thus, the effect of resveratrol on the AGES-induced oxidative stress was investigated in RIN-5F cells. RIN-5F cells that had been adhered and pre-cultured with medium alone for 72 h were treated with resveratrol or medium alone for another 3 h. At the end of the culture, the intracellular peroxide level was measured with DCFH-DA fluorescent probe. The fluorescence intensity of the resveratrol-treated group was significantly lower than that of the control (0 μM resveratrol) group (Fig. 4A). This result suggested that resveratrol might reduce oxidative stress in RIN-5F cells. To verify this hypothesis, RIN-5F cells were given oxidative stress by adding AGE1 and AGE2, BSA conjugated with glucose and glyceraldehyde, respectively, to experimental media. Control cells were incubated with BSA alone (CNT) (Fig. 4B). After 3 h treatment with AGES, fluorescence intensity of AGE1 and AGE2-treated cells was significantly higher than that of the CNT-treated cells (Fig. 4 B and E). In the same experiments with AGES, treatment of RIN-5F cells with resveratrol for 3 h resulted in dramatic reductions in oxidative stress with significant differences (Fig. 4 C, D and E). These results clearly defined that resveratrol was capable to protect pancreatic β-cells.
Resveratrol protects pancreatic β-cells from oxidative stress-induced apoptosis. We examined the effect of resveratrol on oxidative stress-induced apoptosis in RIN-5F cells. To quantitatively gain insight into anti-apoptotic effect of resveratrol in AGE2-treated RIN-5F cells, a display of propidium iodide (PI) versus Annexin V-FITC fluorescence was measured by flow cytometric analysis (Fig. 5). Annexin V-positive and PI-negative cells, which are shown in quadrant E4 in Fig. 5 A and B, are thought to be early apoptotic cells.

RIN-5F cells were exposed to 200 μg/ml CNT and AGE2 in the absence or presence of 50 μM resveratrol for 6 h (Fig. 5 A and B). In this experiment, AGE2 was selected, because it induced more notable oxidative stress in RIN-5F cells than did AGE1 (Fig. 4E). The percentage of apoptosis in AGE2-treated cells was significantly higher than that in CNT-treated cells (Fig. 5C). In the same experiments with CNT and AGE2, treatment of RIN-5F cells with resveratrol for 6 h resulted in significant reductions in the percentage of apoptosis (Fig. 5C).

Discussion

Many kinds of phytochemicals have been reported to possess anti-diabetic potentials. Resveratrol has been reported to competent to shift the physiology of mice on a high-calorie diet towards that of mice on a standard diet and improves their survival and insulin sensitivity. This polyphenol also has been found to possess an insulin-mimetic effect in type 1 diabetic model rats and to ameliorate several metabolic parameters. In the present study, resveratrol was demonstrated to suppress the elevation in the blood glucose level and partially improved dyslipidemia in type 2
diabetic model db/db mice. Serum lipid peroxide (TBARS) concentration was significantly lower in the resveratrol group than in the diabetic control group (Fig. 1C). This result suggests that antioxidative property of resveratrol is available in vivo as well as in vitro (Fig. 4).

AMPK is an important protein to provide energy in mammalian cells. Previous reports showed that resveratrol activated AMPK signaling or insulin signaling including Akt. In the present study, we found for the first time that resveratrol promoted rapidly endogenous GLUT4 translocation through simultaneously enhancing phosphorylation of both AMPK (AMPK signaling) and Akt (insulin signaling), resulting in stimulation of glucose uptake in skeletal muscle cells. Although AMPK signaling is independent of insulin signaling, GLUT4 translocation to plasma membrane is a common final event. Thus, resveratrol is suggested to effectively improve and overcome insulin resistance.

Activation of rennin-angiotensin-aldosterone system has been reported to increase ROS and impaired insulin signaling. Transgenic Ren2 rats manifest increased tissue rennin-angiotensin system activity, hypertension and insulin resistance. In the soleus muscle of Ren2 rats, along with increased NADPH oxidase activity and ROS, there was systemic insulin resistance and reduced IRS-1 tyrosine phosphorylation, Akt phosphorylation/activation, and GLUT4 expression, suggesting oxidative stress disturbs insulin signaling in muscle tissues. In isolated cardiomyocytes, elevated levels of lipid peroxidation product 4-hydroxy-2-nonenal (HNE) have been reported to result in the formation of HNE-LKB1 adducts that inhibit LKB1 and hence AMPK activity. Treatment of cardiomyocytes with resveratrol prevents HNE modification of the LKB1/AMPK signaling. If this be true of L6 myotubes and skeletal muscle tissues of db/db mice, oxidative stress is also suggested to disturb AMPK signaling in muscle tissues and to be improved by resveratrol treatment. These two findings are not inconsistent with the results shown in Fig. 3.

In the diabetic state, glycation reaction is observed in various tissues and organs, and various kinds of glycated proteins such as glycosylated hemoglobin, albumin, and lens crystalline are produced in a nonenzymatical manner through the glycation reaction. The reaction produces Schiff base, Amadori product, and finally AGEs. In the present study, the resveratrol group was still in the high blood glucose state in db/db mice, although the polyphenol lowered the level of blood glucose significantly as compared with that of the diabetic control group. Therefore, syntheses of AGEs might occur in both the control and resveratrol groups. Nonetheless, our data demonstrated that resveratrol protected RIN-5F cells from AGEs-induced oxidative stress (Fig. 4) and AGEs-induced apoptosis (Fig. 5), which is followed by decreasing insulin gene expression and secretion. Although not statistically
Fig. 4. Effect of resveratrol on AGEs-induced oxidative stress in RIN-5F cells. Intracellular ROS in RIN-5F cells were measured with DCFH-DA fluorescent probe and a flow cytometer. A: Effect of resveratrol on basal ROS in RIN-5F cells. B: Effects of AGE1 and AGE2 on intracellular ROS levels in RIN-5F cells after 3 h treatment with AGEs. BSA alone (CNT) was used as control. C, D: Effect of resveratrol on AGEs-induced oxidative stress. E: Graph of statistically processed data. Each value represents the mean ± SEM for 4 assays. Values not sharing a common letter are significantly different at p < 0.001 by Tukey-Kramer multiple comparisons test.

Fig. 5. Effect of resveratrol on AGE2-induced apoptosis in cultured RIN-5F cells. The cells were exposed to resveratrol and/or AGE2 for 6 h. BSA alone (CNT) (A) and AGE2 (B) were added to media at a concentration of 200 μg/ml in the absence or presence of 50 μM resveratrol. C: Graph of statistically processed data. Each value represents the mean ± SEM for 3 assays. Values not sharing a common letter are significantly different at p < 0.05 by Tukey-Kramer multiple comparisons test.
significant, the serum insulin level of the resveratrol group tended to increase as compared with that of the diabetic control (Fig. 1C), suggesting that resveratrol might partially rescue exhausted pancreatic β-cells of db/db mice from further AGEs-induced oxidative stress and apoptosis. It is necessary to analyze common signals in vivo such as levels of AGEs, AMPK phosphorylation and GLUT4 translocation in the skeletal muscle of db/db mice to tie the results obtained in vitro in the present study. Further intensive studies from these aspects are required to clarify the precise mechanisms for anti-diabetic action of resveratrol. In a recent study, resveratrol alleviated diabetes mellitus induced vasculopathy through attenuation of RAGE for AGE-NF-κB signaling pathway. Therefore these previous and present experimental results raise the possibility that resveratrol has health benefits as both forceful protector of pancreatic β-cells and soothing agent of hyperglycemia as well as stimulator of glucose uptake by muscle.

In summary, resveratrol was suggested to suppress the elevation in the blood glucose level in db/db mice. This polyphenol showed antioxidative property both in vitro and in vivo. Results obtained also suggested that resveratrol might enhance glucose uptake in the muscle via both AMPK and insulin signaling pathways and reduce the exhaustion of pancreatic β-cells in db/db mice.

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Conflict of Interest

No potential conflicts of interest were disclosed.

Abbreviations

AGEs: advanced glycation end products
AMPK: AMP-activated protein kinase
BSA: bovine serum albumin
CaMKK: calmodulin-dependent protein kinase kinase
DMEM: Dulbecco’s modified Eagle medium
ERK: extracellular signal-regulated kinase
FBS: fetal bovine serum
GLUT4: glucose transporter 4
HNE: 4-hydroxy-2-nonenal
IRS-1: insulin receptor substrate-1
LKB1: liver kinase B1
MAPK: mitogen-activated protein kinase
NADPH oxidase
PI: phosphatidylinositol
PS: phosphatidylserine
RAGE: receptor of AGEs
ROS: reactive oxygen species
TBARS: thiobarbituric acid-reactive substances
T-Ch: total cholesterol
TG: triglyceride

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