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ARTICLE

trans-Resveratrol Inhibits Hyperglycemia-Induced Inflammation and Connexin Downregulation in Retinal Pigment Epithelial Cells

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The purpose of this study was to determine the inhibitory activity of *trans*-resveratrol against hyperglycemia-induced inflammation and degradation of gap junction intercellular communication in retinal pigment epithelial cells. Retinal (ARPE-19) cells were incubated with 5.5 mM glucose, 5.5 mM glucose and 10 μM resveratrol, 33 mM glucose, or 33 mM glucose and 0−10 μM *trans*-resveratrol at 37 °C and 5% CO_2 for 9 days. Cell viability was determined by the crystal violet assay. The levels of low-grade inflammation biomarkers interleukin-6 and interleukin-8 (IL-6 and IL-8), angiogenic factors, and vascular endothelial growth factor (VEGF) were determined by the enzyme-linked immunosorbent assay (ELISA). Gap junction intercellular communication (GJIC) was determined by the scrape-load/dye transfer method. The expression levels of protein kinase Cβ (PKCβ), connexin 43 (Cx43), transforming growth factor-β1 (TGF-β1), and cyclooxygenase-2 (COX-2) were determined by Western blot. Incubation of retinal cells with 10 μM *trans*-resveratrol in the presence of 5.5 mM glucose did not affect any of the biomarkers investigated. Incubation of ARPE-19 cells with 33 mM glucose for 9 days significantly induced the accumulation of VEGF, IL-6, IL-8, TGF-β, and COX-2, activation of PKCβ, and reduction of Cx43 and GJIC. Incubation of ARPE-19 cells with 33 mM glucose in the presence of 0−10 μM *trans*-resveratrol dose-dependently inhibited VEGF, TGF-β1, COX-2, IL-6, and IL-8 accumulation, PKCβ activation, and Cx43 degradation and enhanced GJIC. These data suggest that *trans*-resveratrol can protect the retinal pigment epithelial cells against hyperglycemia-induced low-grade inflammation and GJIC degradation.

KEYWORDS: *trans*-Resveratrol; hyperglycemia; retinal pigment epithelial cells; VEGF; IL-6; IL-8; COX-2; gap junction intercellular communication; connexin 43; PKCβ; TGF-β1

1. INTRODUCTION

Hyperglycemia (≥14 mM glucose) has been associated with the pathogenesis of vascular complications in diabetes, including the breakdown of the blood retinal barrier (BRB) (*1*−*4*). In retinal pigment epithelial (ARPE-19) cells, hyperglycemia induces the accumulation of transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9), disrupts and downregulates gap junction intercellular communication (GJIC), activates protein kinase C (PKC), and creates conditions that favor increased degradation of connexin 43 (Cx43) (*2*−*7*).

The BRB prevents the entrance of toxic molecules, water, and plasma proteins to the retina and restricts the passage of systematically applied drugs. The integrity of the BRB is essential for maintaining normal visual function (*8*). The retinal pigment epithelium (RPE) cells form the outer BRB, and the retinal vascular endothelium forms the inner BRB. In an inflammatory situation, such as chronic hyperglycemia, the slowly progressive degradation of RPE cells leads to slowly progressive degradation of BRB and central vision loss. Gap junctions (GJs) are intercellular channels, composed of proteins called connexins (Cx); in most cells, they permit neighboring cells to communicate directly by sharing small cytoplasmic molecules, such as ions and second messengers. Connexins are identified by the molecular mass in kilodaltons of their polypeptide chain (e.g., Cx26, Cx32, and Cx43) (*2*). Cx43 is abundantly present in the retinal vascular cells, where it maintains intercellular communication and most likely helps maintain the integrity of the BRB. As a result, downregulation of Cx43 expression and reduced GJIC may lead to disruption of the BRB, disruption of homeostasis between retinal vascular cells, and development of cataracts (*3*). To maintain homeostasis, a delicate balance between the number of cells undergoing apoptosis and cells proliferating must be maintained or provided. Between 98 and 99% of ophthalmic drugs applied systemically remain in the plasma, whereas only 1−2% reach the ocular tissues because of the limited blood flow and the blood ocular barriers. As a result, large concentrations of drugs are left in the body with subsequent unwanted side effects. The retinal vessels are however permeable to lipophilic substances. The identification of health-enhancing dietary bioactive compounds

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that can cross the BRB at physiological concentration could lead to the development of dietary interventions for the prevention of hyperglycemia- and oxidative-stress-induced degradation of retinal cells.

trans-Resveratrol (3,4′,5-trihydroxystilbene) is a phytoalexin found in wine grapes infected by the fungus *Botrytis cinerea* previous to vintage, mulberries, plums, peanuts and some other nuts, red wines, and to a lesser extent, white wines. The best sources of resveratrol among red wines are Pinot Noir, regardless of their origin. Resveratrol exists as a *cis* or *trans* isomer, and the *trans* isomer is relatively more stable than the *cis* form. The literature is well-established that *trans*-resveratrol *in vitro* and in animal models exerts cardiovascular and prolonged aging benefits (*9*−*14*). The French paradox, an apparent correlation that inversely links regular red wine consumption and a high fat diet by French people with a low incidence of cardiovascular disease, has been associated with the presence of *trans*-resveratrol in red wines, although the contribution of other phenolics may also play into action (*15*). *trans*-Resveratrol has also been associated with protection against a variety of age-related diseases, including cancer, diabetes, Alzheimer, cardiovascular, and pulmonary diseases (*14*, *16*). However, data on *trans*-resveratrol interactions with retinal cells are limited. *trans*-Resveratrol at 50−100 μmol/L significantly reduced hydrogen-peroxide-induced oxidation, proliferation, and apoptosis of RPE cells by 10 and 25%, without toxicity to the RPE cells (*17*). *trans*-Resveratrol at 40 mM quenched singlet oxygen species associated with photoexcitation of A2E, one of the most prominent age-related hydrophobic pigments (lipofuscin) in retinal pigment epithelial cells and a biomarker of age-related macular degeneration (*18*). In view of recent reports that associate *trans*-resveratrol with prolonged healthy life, the objective of this research was to study the inhibitory effect of *trans*-resveratrol against hyperglycemia-induced oxidative stress and downregulation of Cx43 and reduced GJIC in ARPE-19 cells.

2. MATERIALS AND METHODS

2.1. Materials. Food-grade *trans*-resveratrol was purchased from Chromadex (Irvine, CA). Glucose, crystal violet, and Lucifer Yellow were from Sigma (St. Louis, MO). Rabbit anti-PKCβ polyclonal antibody was obtained from Signalway Antibody, Ltd. (Pearland, TX). Mouse anti-COX-2 monoclonal antibody was obtained from Cayman Chemicals (Ann Arbor, MI). Mouse anti-TGF-β1 and goat anti-Cx43 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kit was purchased from eBioscience (San Diego, CA). The interleukin-8 (IL-8) ELISA kit was obtained from BioLegend (San Diego, CA). Rhodamine dextran, electrophoresis gels, and Western blot kits (WesternBreeze) were purchased from Invitrogen (Carlsbad, CA). The *DC* protein assay kit was from Bio-Rad (Hercules, CA). All other reagents were of analytical grade.

2.2. Cell Culture. Human retinal pigment epithelial cells [ARPE-19, American Type Culture Collection (ATCC) CRL-2302] within 10 passages from the time of purchase were maintained as suggested by ATCC using a 1:1 mixture of Dulbecco's modified Eagle's medium and HAM F-12 nutrient mixture supplemented with 10% fetal bovine serum at 37 °C, 5% CO_2, and 90% relative humidity. Confluent cells (90% confluence) were used throughout the experiments.

2.3. Cell Viability Assay. ARPE-19 cells (5.0×10^5 cells/well) were seeded in triplicates in 24-well plates and incubated with serum-free medium containing 5.5 mM glucose (mimicking physiological conditions), 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients), 5.5 mM glucose and 10 μM *trans*-resveratrol, or 33 mM glucose and 0−10 μM *trans*-resveratrol at 37 °C, 5% CO_2, and 90% relative humidity. The medium containing 5.5 mM glucose, 33 mM glucose, or 33 mM glucose and 0−10 μM *trans*-resveratrol and the cells was incubated for 9 days. Cell viability was evaluated by the crystal violet assay as follows. At the end of the incubation period, the plate was stained with 0.5% crystal violet in 20% methanol, rinsed with deionized distilled water, and air-dried. The crystal violet stain was dissolved in a solution of 0.1 M sodium citrate and ethanol (1:1). The absorbance was read at 540 nm using an ELISA plate reader (Spectra Max Plus, Molecular Devices, Sunnyvale, CA). Each concentration of the inhibitor and blank was carried out in triplicate. Data were normalized to levels in untreated control cells and plotted as a percentage of the control cell viability.

2.4. Determination of VEGF, IL-6, and IL-8 Levels in ARPE-19 Cells. The levels of VEGF in the supernatants were measured by ELISA using a commercial kit from Peprotech (Rocky Hill, NJ). The levels of IL-6 in the supernatants were determined by ELISA using a commercially available kit from eBioscience (San Diego, CA). The levels of IL-8 in the supernatants were determined by ELISA using a commercially available kit from BioLegend (San Diego, CA).

2.5. Scrape-Loading/Dye Transfer Assay of GJIC. The scrape-loading/dye transfer method was followed with minor modifications (*19*). Briefly, ARPE-19 cells (1.0×10^6 cells/well) seeded in 6-well plates were treated with 5.5 mM glucose, 33 mM glucose, or 33 mM glucose and different concentrations of *trans*-resveratrol at 37 °C and 5% CO_2 for 9 days. At the end of the incubation period, the cells were rinsed 3 times with Hanks' balanced salt solution containing 1% bovine serum albumin (HB). The HB was removed, and 1 mL of phosphate-buffered saline (PBS) containing 0.25% Lucifer Yellow (LY) and 0.75% of the GJ-impermeant compound rhodamine dextran (fluoro-ruby; molecular mass, 10 kDa; Molecular Probes) was applied to the center of the plate. A scalpel was used to create three scrape lines across the cell monolayer. Cells were incubated at 37 °C and 5% CO_2 for 5 min and then quickly rinsed 3 times with HB prior to 5 min of incubation in HB at room temperature to allow dye transfer. The cells were rinsed 3 times with PBS and immediately fixed for 30 min at room temperature with 3.75% paraformaldehyde. Dye transfer was examined by fluorescence microscopy (Zeiss Axiovert 405 M).

2.6. Western Blot Analysis. ARPE-19 cells incubated with or without *trans*-resveratrol were washed twice with PBS (pH 7.4), scraped, and treated with RIPA lysis buffer [50 mM Tris-HCl at pH 7.4, 150 mM NaCl, 0.1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS)] supplemented with protease inhibitor mixture, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM sodium orthovanadate. After 30 min of incubation on ice, the lysates were vortexed and centrifuged at 15000*g* for 30 min at 4 °C. The supernatants were removed, and protein concentrations of the lysate supernatants were determined using the *CD* protein assay (Bio-Rad, Hercules, CA). Equal protein levels (50 μg) were separated by electrophoresis using 12% Bis-Tris gels and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS)/Tween 20 (0.05%), followed by incubation with a primary antibody (Cx43, COX-2, TGF-β1, PKCβ, or β-actin) in 5% nonfat dry milk in TBS/Tween 20. Visualization of the bound primary antibody was performed by probing with alkaline phosphatase-conjugated secondary antibody and exposure to chromogenic detection reagent. Results are representative of three separate experiments. The density of each band was analyzed using the NIH Image J 1.41 analysis program.

2.7. Statistical Analysis. Results are mean ± standard deviation (SD), and differences between the control and resveratrol treatment were analyzed by analysis of variation (ANOVA). Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1. Effect of *trans*-Resveratrol on ARPE-19 Cell Viability. ARPE-19 cells incubated with 5.5 mM glucose, 5.5 mM glucose and 10 μM *trans*-resveratrol, 33 mM glucose, or 33 mM glucose and 0−10 μM *trans*-resveratrol remained viable for the 9 days of incubation time (**Figure 1**). The viability of ARPE-19 cells treated with *trans*-resveratrol was not significantly different from the viability of control untreated ARPE-19 cells incubated in 5.5 mM glucose ($p < 0.05$).

3.2. Effect of *trans*-Resveratrol on Hyperglycemia-Induced Secretion of VEGF, IL-6, and IL-8 in ARPE-19 Cells. Hyperglycemia caused an increase in the levels of VEGF in the conditioned media of ARPE-19 cells (**Figure 2**). The expression of the

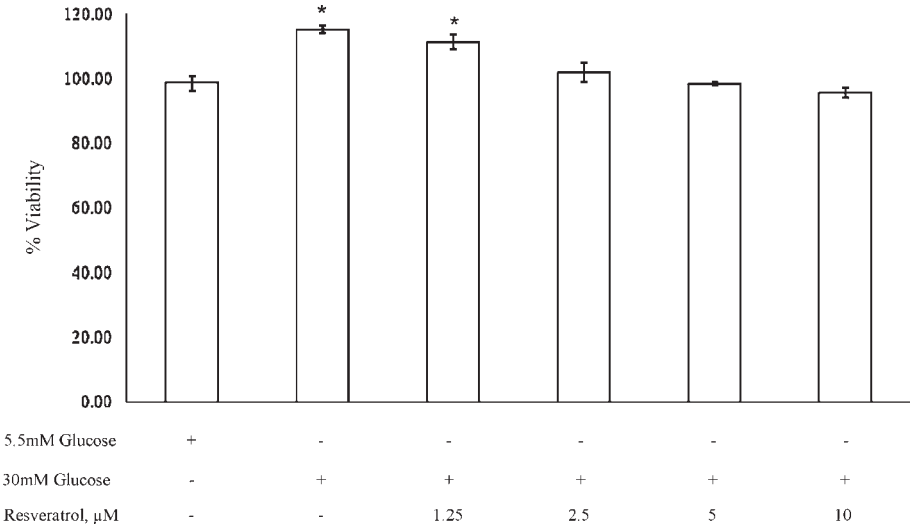

Figure 1. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on retinal pigment epithelial cell (ARPE-19) proliferation. ARPE-19 cells were incubated in medium containing 5.5 mM glucose (mimicking physiological conditions) and 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients) in the absence or presence of increasing concentrations of *trans*-resveratrol. Viability assay was performed using the crystal violet assay. Results are averages of at least three experiments carried out in triplicate. (∗) $p < 0.05$ is considered significantly different from the control.

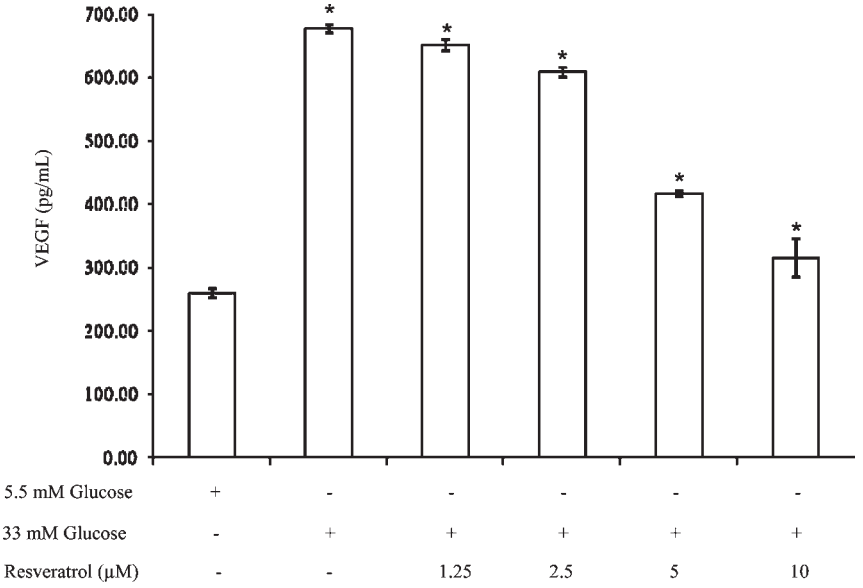

Figure 2. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on the levels of VEGF in retinal pigment epithelial (ARPE-19) cells. ARPE-19 cells were incubated in medium containing 5.5 mM glucose (mimicking physiological conditions) and 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients) in the absence or presence of increasing concentrations of *trans*-resveratrol. VEGF levels in the supernatants were measured by ELISA. Results are averages of at least three experiments carried out in triplicate. (∗) $p < 0.05$ is considered significantly different from the control.

angiogenic factor VEGF increases the expression of the inflammatory biomarker IL-6. Hyperglycemia also caused an increase in the levels of IL-6 in the conditioned media of ARPE-19 cells (**Figure 3**). *trans*-Resveratrol dose-dependently inhibited the increase in VEGF levels in hyperglycemic ARPE-19 cells. Control ARPE-19 cells secreted 699.94 pg/mL of IL-6, whereas ARPE-19 cells treated with 33 mM glucose secreted 1965.71 pg/mL of IL-6 (**Figure 3**). *trans*-Resveratrol reduced the IL-6 level in 33 mM glucose-treated retinal cells from 1965.71 to 793.30 pg/mL (**Figure 3**). The level of IL-8 in the supernatants from control retinal cells was lower than the level of IL-8 in 33 mM glucose-treated retinal cells (**Figure 4**). *trans*-Resveratrol dose-dependently reduced the level of IL-8 in retinal cells treated with 33 mM glucose. Both IL-6 and IL-8 are considered biomarkers of low-grade inflammation, and our results suggest that *trans*-resveratrol inhibited low-grade inflammation caused by hyperglycemia in ARPE-19 cells maintained under hyperglycemic conditions for 9 days.

3.3. Effect of *trans*-Resveratrol on GJIC. The scrape-loading/dye transfer assay allows for simultaneous monitoring of dye coupling in a large population of cells (*20*). Cell communication was maintained in control cells, as shown by the level of GJIC among cells (**Figure 5A**). Cell communication was low or lost in cells treated with 33 mM glucose, as shown by the layers of cells on both sides of the scratch (**Figure 5B**). The GJIC decreased in ARPE-19 cells treated with 33 mM glucose for 9 days compared to control cells in 5.5 mM glucose for the same duration. *trans*-Resveratrol dose-dependently enhanced GJIC activity in ARPE-19 cells treated with 33 mM glucose and prevented GJIC loss, as shown by the layers of cells on both sides of the scratch (panels **C**−**E** of **Figure 5**). Hyperglycemia leads to a decrease in GJIC by a

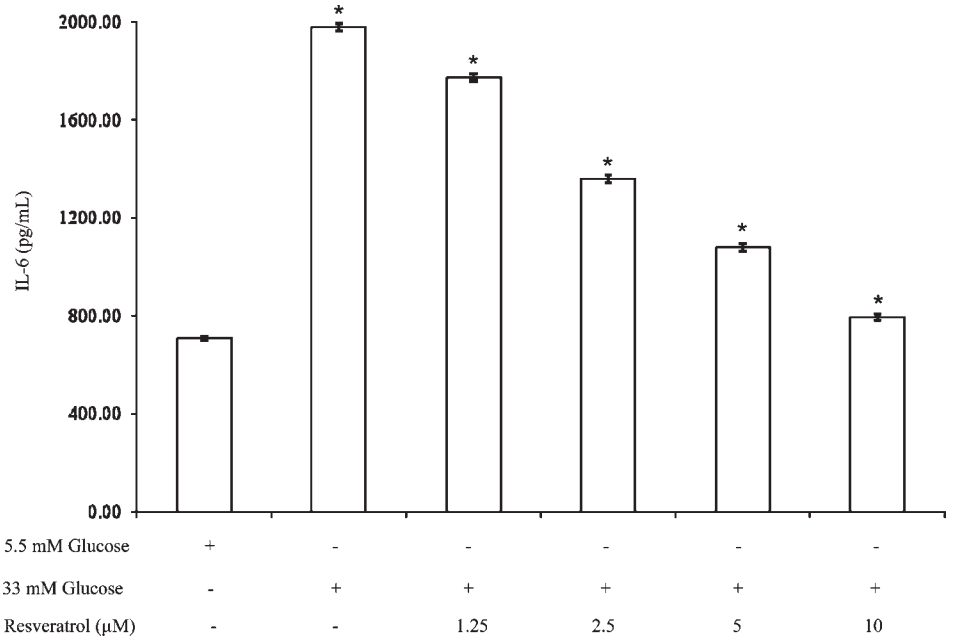

Figure 3. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on the levels of IL-6 in retinal pigment epithelial (ARPE-19) cells. ARPE-19 cells were incubated in medium containing 5.5 mM glucose (mimicking physiological conditions) and 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients) in the absence or presence of increasing concentrations of *trans*-resveratrol. IL-6 levels in the supernatants were measured by ELISA. Results are averages of at least three experiments carried out in triplicate. (∗) $p < 0.05$ is considered significantly different from the control.

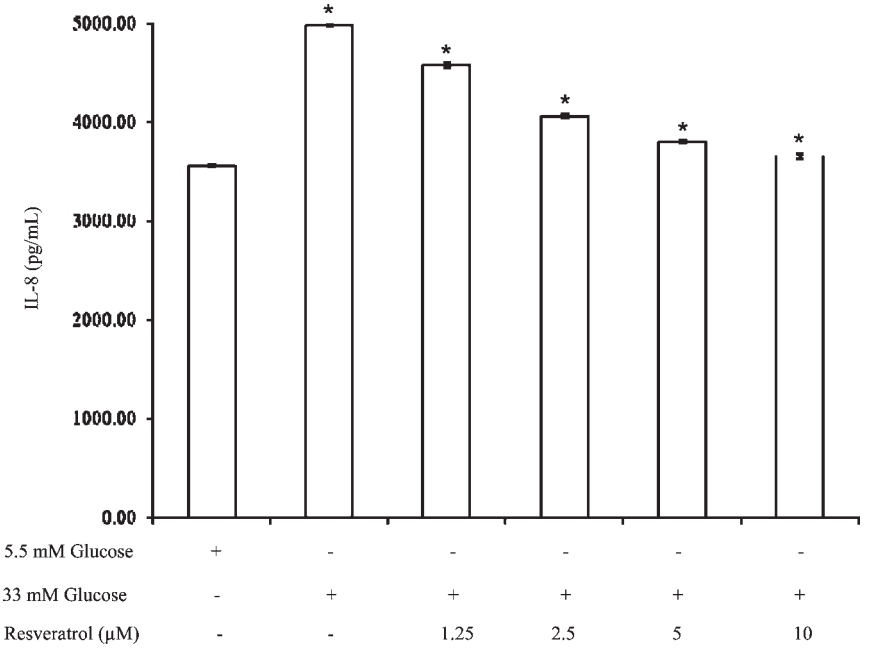

Figure 4. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on the levels of IL-8 in retinal pigment epithelial (ARPE-19) cells. ARPE-19 cells were incubated in medium containing 5.5 mM glucose (mimicking physiological conditions) and 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients) in the absence or presence of increasing concentrations of *trans*-resveratrol. IL-8 levels in the supernatants were measured by ELISA. Results are averages of at least three experiments carried out in triplicate. (∗) $p < 0.05$ is considered significantly different from the control.

proteasome-dependent mechanism (*21*). To ascertain that *trans*-resveratrol enhanced GJIC in high glucose cells by inhibiting proteasome activity, we measured the levels of PKCβ in 5.5 mM glucose, 33 mM glucose, and 33 mM glucose and *trans*-resveratrol-treated retinal cells and the results are presented in the western blot section below.

3.4. Effect of *trans*-Resveratrol on Cx43 Downregulation and COX-2, TGF-β1, and PKCβ Activation. Hyperglycemia has been shown to downregulate Cx43 (*22*). To determine whether *trans*-resveratrol inhibited hyperglycemia-induced reduced GJIC activity in ARPE-19 cells, we performed Western blot analysis to determine Cx43 protein levels in control and *trans*-resveratrol-treated retinal cells. High glucose (33 mM) induced a decrease in Cx43 levels, and the observed decrease in Cx43 levels was dose-dependently prevented by *trans*-resveratrol (**Figure 6**). Cx43 degradation leads to a decrease of GJIC. *trans*-resveratrol dose-dependently restored Cx43 to levels comparable to control cells. Incubation of ARPE-19 cells at hyperglycemic concentrations

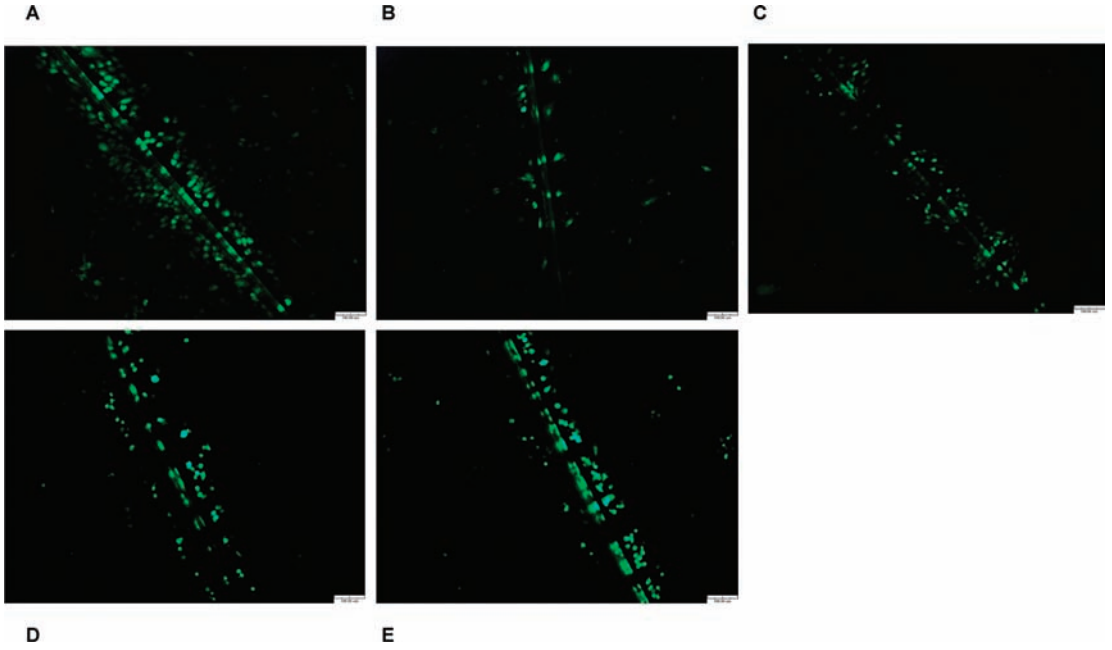

Figure 5. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on the activity of GJIC in retinal pigment epithelial (ARPE-19) cells. (A) Control ARPE-19 cells in 5.5 mM glucose (mimicking physiological conditions), (B) ARPE-19 cells incubated with 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients), (C) ARPE-19 cells incubated with 33 mM glucose and 1.25 μM *trans*-resveratrol, (D) ARPE-19 cells incubated with 33 mM glucose and 5 μM *trans*-resveratrol, and (E) ARPE-19 cells incubated with 30 mM glucose and 10 μM *trans*-resveratrol. Results are representatives of three independent experiments. (*) $p < 0.05$ is considered significantly different from the control.

caused a significant increase of the COX-2 level (**Figure 6**). Results in **Figure 6** also show that the observed increase in COX-2 was dose-dependently inhibited by *trans*-resveratrol. Hyperglycemia increased the level of TGF-β1 in ARPE-19 cells, and *trans*-resveratrol dose-dependently reversed the activation of the enzyme. Results in **Figure 6** also indicate that PKCβ activity was higher in 33 mM glucose-treated ARPE-19 cells compared to control cells. Remarkably, PKCβ activity was reduced in *trans*-resveratrol-treated retinal cells in the presence of 33 mM glucose cells than retinal cells in 33 mM glucose without *trans*-resveratrol.

4. DISCUSSION

In the present study, we demonstrated for the first time the inhibitory effect of resveratrol on hyperglycemia-induced retinal pigment epithelial cell inflammation together with underlying molecular mechanisms. *trans*-Resveratrol ameliorated hyperglycemia-induced parameters, including decreased GJIC, secretion of cytokines IL-6 and IL-8, downregulation of Cx43, and activation of TGF-β, PKCβ, and COX-2 (**Figures 3**, **4**, and **6**). *trans*-Resveratrol is a phytoalexin contained abundantly in red wine, mulberry, grapes, and peanut skins. Several biological activities, including anti-angiogenic, anti-tumorigenic, antioxidative, neuroprotective activities, have been ascribed to resveratrol (*14*, *22*). *trans*-Resveratrol inhibits COX-2 activation, which is one of the key steps in the arachidonic acid cascade-induced inflammation (*23*). Consistent with other studies that have demonstrated the anti-inflammatory activity of *trans*-resveratrol on several chronic diseases, the present data demonstrate that resveratrol has an inhibitory effect on hyperglycemia-induced inflammation in retinal pigment epithelial cells. Repeated administration of *trans*-resveratrol at a dose of 20 mg/kg of body weight to rats for 28 days showed no biochemical, hematological, or pathological differences compared to the control untreated animal group (*24*). Resveratrol is bioavailable, not toxic, and found in serum as a glucuronide (*25*, *26*). The levels of *trans*-resveratrol stored in retinal tissues is not known, but because *trans*-resveratrol is bioavailable and not toxic to retinal cells at levels as high as 40 mM (*18*, *27*) and capable of crossing the blood brain barrier, it is therefore likely that bioavailable *trans*-resveratrol or its metabolites can reach the retinal tissues and provide the much purported protection against hyperglycemia-induced retinal cell injury. Hyperglycemia induced TGF-β1 expression in ARPE-19 cells by activation of the ERK/MAPK/Akt pathway in human RPE cells (*2*). TGF-β1 expression leads to upregulation of VEGF, and in turn, VEGF induces both retinal neovascularization and fibrosis around these new vessels in diabetic complications (*28*−*30*). In this study, *trans*-resveratrol reduced the expression of TGF-β1 and VEGF in retina cells under hyperglycemic conditions. Hyperglycemia induces the secretion of IL-6 and IL-8, and the secretion of both cytokines indicates an early event of inflammation. The levels of IL-6 and IL-8 in hyperglycemic ARPE-19 cells increased in comparison to the control cells. *trans*-Resveratrol dose-dependently reduced IL-6 and IL-8 in retinal cells incubated in hyperglycemic media. *trans*-Resveratrol inhibited IL-6 gene expression and protein secretion in mixed glial cultures under hypoxia/hypoglycemia, followed by reoxygenation (*31*). Results presented here indicate that, under hyperglycemic conditions, *trans*-resveratrol was effective in inhibiting IL-6 secretion by hyperglycemic retinal cells. IL-8 is a potent proinflammatory cytokine. Hyperglycemia induces the release of cytokines, which in turn lead to retinal cell injury, suggesting that hyperglycemia is linked to the pathogenesis of diabetes retinopathy (*32*). Treatment options for diabetes retinopathy include achieving and maintaining a tight glucose control in tissues or retina ablation. Glucose control is very challenging, and retina ablation is currently the only therapy for direct treatment of proliferative diabetic retinopathy (PDR). Hyperglycemia induces overexpression of COX-2. The level of COX-2 in ARPE-19 cells incubated with 33 mM glucose was reduced in the presence of *trans*-resveratrol. PKCβ is activated by hyperglycemia, and PKCβ in turn may act in concert with hypoxia to upregulate VEGF, with the latter binding to its kinase domain

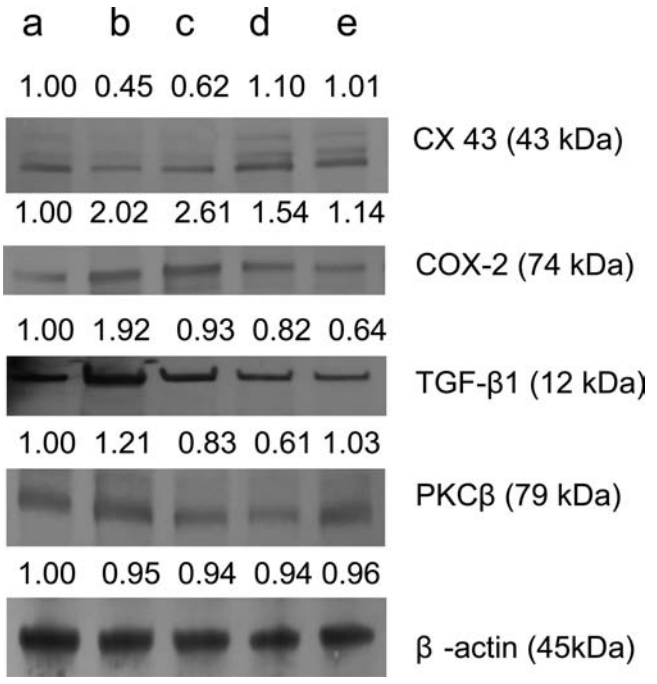

Figure 6. Effect of hyperglycemia in the absence or presence of *trans*-resveratrol on the levels of COX-2, TGF-β1, CX43, and PKCβ in retinal pigment epithelial (ARPE-19) cells. Cell lysates were separated by western blot, transferred on PVDF membranes, incubated with appropriate primary antibodies, followed by incubation with appropriate secondary antibodies conjugated to alkaline phosphatase, and visualized using chromogenic detection. The observed protein density was normalized to the control cells containing 5.5 mM glucose (mimicking physiological conditions). (a) 5.5 mM glucose, (b) 33 mM glucose, (c) 33 mM glucose (mimicking the hyperglycemia that occurs in diabetic patients) and 1.25 μM *trans*-resveratrol, (d) 33 mM glucose and 8 μM *trans*-resveratrol, and (e) 33 mM glucose and 10 μM *trans*-resveratrol. Results are representatives of three independent experiments. Numbers on top of each lane represent the fraction of band intensity compared to the control, which is represented by lane a.

receptor and activating a variety of molecules that may initiate the breakdown of the BRB (*33*). High extracellular glucose concentrations promote the activation of PKC because of *de novo* synthesis of diacylglycerol (DAG) induced by hyperglycemia in tissues such as the retina *in vitro* and *in vivo* (*34*−*37*). PKCβI and PKCβII isoforms, which are activated by DAG, are present at high concentrations in the retina and promote the synthesis of VEGF. Inhibition of PKCβ activation by *trans*-resveratrol may prevent the progression of hyperglycemia-induced retinal cell degradation. PKC was inhibited by *trans*-resveratrol with IC_{50} = 2 μM (*38*). Hyperglycemia has shown to reduce GJIC in retinal, endothelial, and smooth muscle cells (*3*). Hyperglycemia can also induce a decreased in Cx43 expression, as shown in **Figure 5**, which is associated with uncontrolled proliferation of ARPE-19 cells (*3*,*39*). *trans*-Resveratrol protected ARPE-19 cells from the decrease in Cx43 caused by hyperglycemia. Uncontrolled proliferation of ARPE-19 cells is observed in diabetes mellitus and retinitis pigmentosa and has been associated with decreased GJIC in retinal pigment epithelial cells (*2*). GJIC is recognized as the only means for direct contact between cytoplasms of adjacent animal cells, and disturbances of GJIC has been associated with many pathological conditions, including cancer, macular degeneration, and hereditary diseases (*40*). Bioactive compounds that can improve GJIC among cells may delay the onset of some chronic degenerative diseases. For instance, vitamin D3, cholecalciferol, at concentrations between 0.01 and 1 μM induced cell−cell communication via gap junctions in murine fibroblasts (C3H/10T 1/2 cells), and this property may contribute to the antiproliferative effects of vitamin D exhibited in some types of cancer (*41*). Similarly, dietary bioactive compound Bowman−Birk inhibitor, which has been shown to act as a chemopreventive agent in several types of tumor cells, suppressed U2OS growth by inducing the expression of Cx43 (*42*). The effects of hyperglycemia on ARPE-19 cells have been demonstrated *in vitro* as well as in several clinically relevant animal models of retinopathy and in humans. Because of its location, ARPE-19 cells play a key role in the pathological process that lead to vision loss. The results of our *in vitro* study suggest that *trans*-resveratrol may protect retinal cells against high glucose by reducing low-grade inflammation and attenuating the increase in pro-angiogenic biomarkers that stimulate the degradation of retinal cells by high glucose. It is suggested that *in vivo* studies be performed to determine the health benefits of resveratrol in retinal cells.

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