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All-trans Retinoic Acid Attenuates High Glucose-Induced VEGFA Expression via Inhibition of p38 MAPK and NF-κB in ARPE-19 Cells

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Abstract

Diabetic retinopathy (DR), a leading cause of vision loss, is characterized by retinal inflammation, vascular leakage, and pathological neovascularization, with vascular endothelial growth factor A (VEGFA) playing a central role in its progression. While anti-VEGF therapies are effective, their invasive nature and associated risks emphasize the need for safer and more accessible alternatives. This study aimed to investigate the potential of all-trans retinoic acid (RA), a bioactive metabolite of vitamin A, to suppress high glucose-induced VEGFA expression in retinal pigment epithelial (ARPE-19) cells and explore the underlying molecular mechanisms. ARPE-19 cells were treated with high glucose (30 mM) in the presence or absence of RA (5 or 20 μM). Cell viability was assessed by CCK-8 assay, while VEGFA mRNA and protein levels were measured using quantitative real-time PCR and ELISA, respectively. The activation of p38 MAPK and nuclear translocation of NF-κB p65 was evaluated through Western blot analysis. RA treatment significantly reduced high glucose-induced VEGFA expression at both the mRNA and protein levels, without affecting cell viability. Mechanistically, RA inhibited the phosphorylation of p38 MAPK and the nuclear translocation of NF-κB p65, suggesting that these pathways contribute to VEGFA regulation under hyperglycemic conditions. These findings highlight the anti-inflammatory and anti-angiogenic effects of RA in ARPE-19 cells and propose RA as a potential, safe, and non-invasive therapeutic candidate for the early intervention of diabetic retinopathy. Further in vivo studies are needed to validate its clinical applicability.

Keywords: Retinoic acid (RA), VEGFA, Diabetic retinopathy (DR), p38 MAPK, NF-κB

Introduction

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes mellitus and remains a leading cause of preventable vision loss worldwide [1]. Chronic hyperglycemia triggers a cascade of pathological and innate immunological alterations in the retina, including oxidative stress, inflammation, and vascular dysfunction [2]. A key consequence of these processes is the upregulation of vascular endothelial growth factor A (VEGFA), which plays a central role in DR pathogenesis [3,4]. VEGFA promotes endothelial cell proliferation, increases vascular permeability, disrupts the blood-retinal barrier (BRB), and drives aberrant neovascularization—features that are closely associated with disease progression from non-proliferative to proliferative DR [5]. Numerous studies have shown that VEGFA levels are markedly elevated in the vitreous and retinal tissues of patients with DR, and its expression correlates with the severity of retinal edema and neovascularization [6,7]. As such, VEGFA has become a primary therapeutic target in DR management. Anti-VEGF agents, such as ranibizumab and aflibercept, have been widely adopted in clinical practice and have demonstrated efficacy in reducing VEGF-mediated vascular leakage and macular edema [8]. However, these treatments are invasive, require frequent intravitreal injections, and carry risks of adverse effects, highlighting the need for safer, more accessible, and cost-effective strategies to

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regulate VEGFA expression and mitigate early retinal damage in diabetes.

At the molecular level, prolonged hyperglycemia activates several intracellular signaling pathways that contribute to the development and progression of diabetic retinopathy. Among these, the mitogen-activated protein kinase (MAPK) pathway—particularly the p38 MAPK branch—and the nuclear factor kappa B (NF-κB) pathway are critically involved in mediating the cellular response to oxidative stress and inflammation [9–12]. Activation of p38 MAPK under high glucose conditions leads to the phosphorylation of transcriptional regulators and pro-inflammatory mediators, resulting in the upregulation of genes such as VEGFA and interleukin-6 (IL-6) [13,14]. Concurrently, NF-κB, a key transcription factor involved in inflammatory signaling, is activated via the translocation of its p65 subunit into the nucleus, where it promotes the transcription of a broad range of pro-angiogenic and pro-inflammatory genes [15]. Both p38 phosphorylation and p65 nuclear translocation have been widely implicated in the exacerbation of retinal vascular dysfunction and neovascularization observed in diabetic retinopathy [16]. Given their upstream regulatory role in VEGFA expression, targeting these pathways offers a promising therapeutic approach to mitigate retinal inflammation and vascular leakage in the early stages of the disease.

Retinoic acid (RA), a bioactive metabolite of vitamin A, is well recognized for its regulatory roles in cell proliferation, differentiation, and immune modulation. In recent years, accumulating evidence has highlighted its potential as an anti-inflammatory and anti-angiogenic agent in various disease models [17,18]. RA may modulate the expression of key pathological mediators, including VEGFA and matrix metalloproteinases (MMPs), through its ability to interact with nuclear retinoic acid receptors (RARs) and alter transcriptional activity [19,20]. These findings suggest that RA may interfere with upstream signaling pathways implicated in diabetic retinopathy, such as NF-κB and MAPKs. Compared to conventional anti-VEGF biologics, RA offers several advantages: it is a naturally derived compound, widely available, and generally considered to have low toxicity. Importantly, RA is a well-characterized therapeutic agent. While its systemic use requires careful monitoring, its potential for repurposing in localized ocular therapies remains an attractive avenue for investigation.

Given the central role of VEGFA in diabetic retinopathy and the involvement of p38 MAPK and NF-κB signaling in its transcriptional regulation, identifying upstream modulators that can safely suppress this axis is of therapeutic interest [13,14]. While previous studies have suggested the regulatory effects of retinoic acid on angiogenic and inflammatory pathways [18]. Its specific role in modulating VEGFA expression and related intracellular signaling in retinal pigment epithelial cells under hyperglycemic conditions remains poorly understood. Therefore, the present study aimed to investigate whether all-trans retinoic acid (RA) could suppress high glucose-induced VEGFA expression in ARPE-19 cells and to elucidate the underlying mechanisms involving the p38 MAPK and NF-κB signaling pathways. We hypothesized that RA treatment would attenuate VEGFA expression by inhibiting the phosphorylation of p38 MAPK and the nuclear translocation of NF-κB p65, thereby mitigating key pathogenic events triggered by hyperglycemia in retinal epithelial cells. This study may provide new insights into the potential application of RA as an adjunctive or alternative strategy for the early management of diabetic retinopathy.

Materials-Methods

Reagents and Materials

Dulbecco's Modified Eagle Medium (DMEM)/F-12 and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Other general cell culture reagents were obtained from GIBCO-BRL (Grand Island, NY, USA). All-trans retinoic acid (ATRA; Cat. No. T1051) was purchased from TargetMol (Boston, MA, USA). SYBR® Green PCR Master Mix and the MultiScribe™ Reverse Transcriptase Kit were obtained from Applied Biosystems (Foster City, CA, USA). Unless otherwise specified, all other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture

The human retinal pigment epithelial cell line ARPE-19 (BCRC No. 60383; Bioresource Collection and Research Center, Hsinchu, Taiwan) was used in this study. Cells were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 Nutrient Mixture (Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, and 0.5 mM sodium pyruvate. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% air. The medium was refreshed every 2 to 3 days, and cells were subcultured at a split ratio of 1:3 to 1:5 upon reaching ~80% confluence.

CCK8 assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Cat. No. C0005, TargetMol, Boston, MA, USA) according to the manufacturer's instructions. Briefly, 2×10^3 ARPE-19 cells were seeded into each well of a 96-well plate and treated with various concentrations of all-trans retinoic acid (ATRA; 0, 1, 5, 10, 20, 40, and 80 μM). At 24, 48, 72, or 96 hours post-treatment, 100 μL of 10-fold diluted CCK-8 reagent was added to each well, and the cells were incubated at 37 °C for 2 hours. Absorbance was then measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). All experiments were performed in triplicate. The relative cell viability was calculated as follows: (OD value of ATRA-treated cells / OD value of control cells) × 100%.

Enzyme-Linked Immunosorbent Assay (ELISA)

ARPE-19 cells were seeded into 6-well culture plates at a density of 0.5×10^6 cells per well and allowed to adhere for 24 hours.

The cells were then treated under the following conditions: 5 mM glucose (normal glucose, NG), 30 mM glucose (high glucose, HG), HG supplemented with 5 μM all-trans retinoic acid (ATRA), or HG supplemented with 20 μM ATRA. After 48 hours of incubation, the culture supernatants were collected and centrifuged at 1000 × g for 10 minutes at 4 °C to remove cell debris. The concentration of VEGFA in the supernatants was quantified using a commercial ELISA kit (FineTest, Cat. No. EH0251, Wuhan, China) according to the manufacturer's instructions.

Quantitative Real-Time PCR

Following the manufacturer's instructions, the total RNA was isolated from ARPE-19 cells under various treatment conditions using TRIzol™ reagent (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA synthesis and amplification were performed using the SuperScript™ III One-Step RT-PCR System (Cat. No. 12574026, Thermo Fisher Scientific, Riverstone, NSW, Australia). Quantitative PCR was conducted using SYBR® Green Supermix on an ABI Real-Time PCR Detection System. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 95 °C for 5 seconds and annealing/extension at 60 °C for 30 seconds. The relative mRNA expression of VEGFA was normalized to GAPDH as an internal control and calculated using the 2^−ΔΔCt method. The following primers were used: VEGFA forward 5'-AGGGCAGAATCATCACGAAGT-3' and reverse 5'-AGGGTCTCGATTGGATGGCA-3' (PrimerBank ID: 284172466c1); GAPDH forward 5'-TGTGGGCATCAATGGATTTGG-3' and reverse 5'-ACACCATGTATTCCGGGTCAAT-3' (PrimerBank ID: 126273608c1).

Western Blot Analysis

For whole-cell lysates, cells were lysed in ice-cold RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, and protease/phosphatase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). After incubation on ice for 30 minutes, lysates were centrifuged at 15,000 × g for 10 minutes at 4 °C. Protein concentrations were measured using the BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were denatured by boiling at 95 °C for 10 minutes and resolved on 12% SDS-polyacrylamide gels, followed by transfer to PVDF membranes (Merck Millipore, Burlington, MA, USA).

For nuclear protein extraction, cells were harvested and lysed using a hypotonic lysis buffer (10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and protease/phosphatase inhibitors) and incubated on ice for 15 minutes. NP-40 was added to a final concentration of 0.1%, and cells were vortexed briefly. The nuclear pellet was then resuspended in high-salt nuclear extraction buffer (20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, and protease/phosphatase inhibitors), incubated on ice for 30 minutes with occasional mixing, and centrifuged at 15,000 × g for 15 minutes at 4 °C. The supernatant containing nuclear proteins was collected for further analysis.

The membranes were blocked with 5% non-fat dry milk in TBS-T for 1 hour and incubated overnight at 4 °C with primary antibodies, including anti-phospho-p38 MAPK (Thr180/Tyr182, 1:1000, Cat No. 28796-1-AP, Rabbit IgG), anti-p38 MAPK (1:1000, Cat No. 14064-1-AP, Rabbit IgG), anti-NF-κB p65 (1:1000, Cat No. 10745-1-AP, Rabbit IgG), anti-GAPDH (1:4000, Cat No. 60004-1-Ig, Mouse IgG2b, Clone 1E6D9), anti-α-tubulin (1:1000, Cat No. 11224-1-AP, Rabbit IgG), and anti-histone H1 (1:4000, Cat No. 18201-1-AP, Rabbit IgG), all purchased from Proteintech (Rosemont, IL, USA). After washing, membranes were incubated with HRP-conjugated goat anti-rabbit IgG or goat anti-mouse IgG secondary antibodies (1:4000, Jackson ImmunoResearch, West Grove, PA, USA) for 1 hour at room temperature. The immunoreactive bands were detected using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific) and visualized by chemiluminescence imaging.

Statistical Analysis

All data are presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical significance between groups was evaluated using an unpaired Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant. GraphPad Prism (version X; GraphPad Software, San Diego, CA, USA) was used for data analysis and figure generation.

Results

1. Retinoic acid reduces ARPE-19 cell viability in a time- and dose-dependent manner

To evaluate the cytotoxicity of RA, ARPE-19 cells were treated with various concentrations of all-trans retinoic acid (0, 1, 5, 10, 20, 40, and 80 μM) and incubated for 0, 24, 48, 72, or 96 hours. Cell viability was assessed using the CCK-8 assay. As shown in Figure 1, RA at concentrations above 20 μM progressively reduced cell viability in a dose- and time-dependent manner. The most significant reductions were observed at 40 and 80 μM after 72 and 96 hours of treatment. These results indicate that high concentrations of RA may inhibit ARPE-19 cell proliferation or induce cytotoxicity.

2. Retinoic acid inhibits high glucose-induced VEGFA expression in ARPE-19 cells

To investigate the effect of RA on angiogenic signaling, VEGFA expression was evaluated under high glucose (HG) conditions. ARPE-19 cells were exposed to 30 mM glucose for 48 hours, with or without co-treatment of RA at 5 or 20 μM. The secreted VEGFA protein levels in the culture supernatants were measured by ELISA, and mRNA expression was assessed using quantitative real-time PCR (qPCR). As shown in Figure 2, both VEGFA protein and mRNA levels were significantly elevated following high glucose exposure, while RA treatment dose-de-

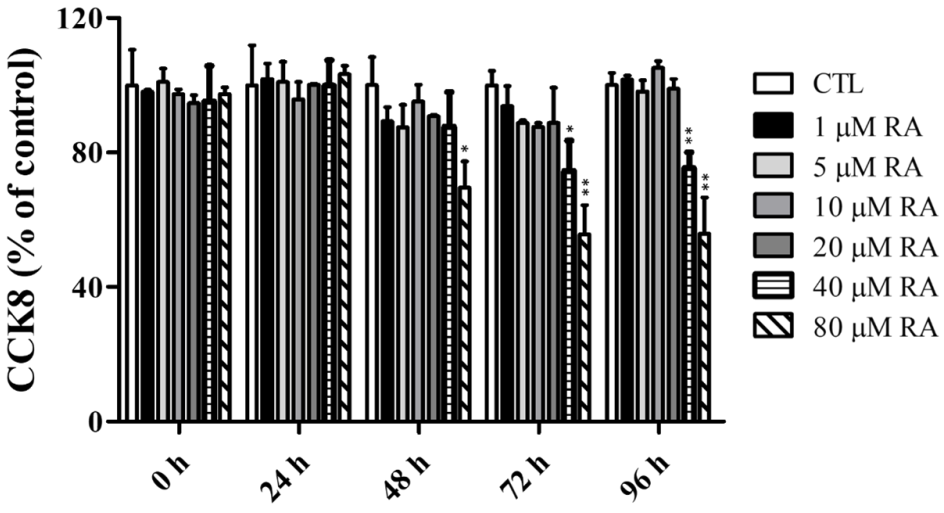

Figure 1. Effects of different concentrations of RA on ARPE-19 cell viability.
ARPE-19 cells were treated with various concentrations of RA (0, 1, 5, 10, 20, 40, and 80 μM) for 0, 24, 48, 72, or 96 hours. Cell viability was assessed at each time point using the CCK-8 assay. Data are expressed as mean ± SD from three independent experiments. Statistical significance was determined using Student's *t*-test. $P < 0.05$, $P < 0.01$ compared with the untreated control at each corresponding time point.

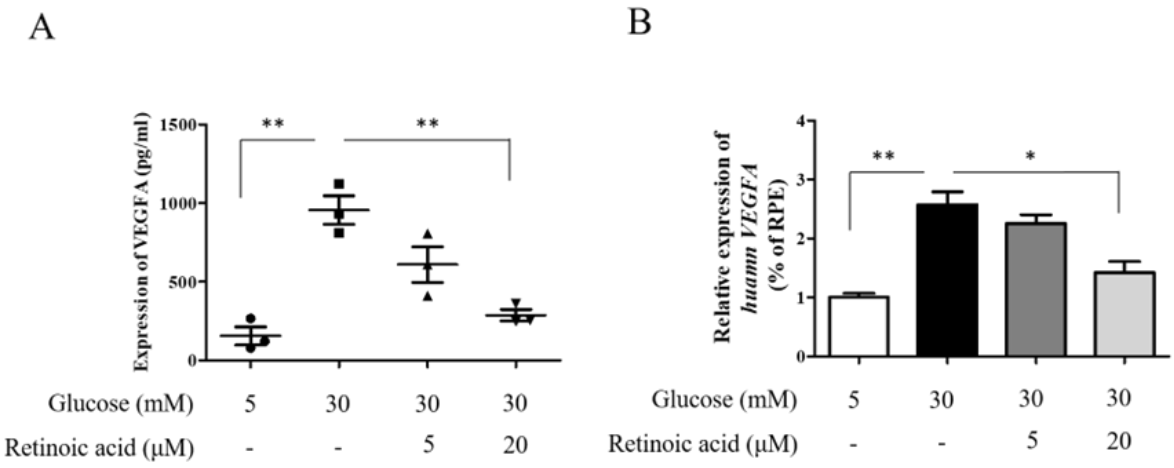

Figure 2. RA inhibits high glucose-induced VEGFA expression in ARPE-19 cells.
ARPE-19 cells were treated with 30 mM high glucose in the presence or absence of RA (5 or 20 μM) for 48 hours. (A) The concentration of VEGFA in the culture supernatants was measured using an ELISA kit. (B) The mRNA expression of VEGFA was determined by qPCR after total RNA extraction. Data are presented as mean ± SD from three independent experiments. Statistical significance was determined using Student's *t*-test. $P < 0.05$, $P < 0.01$ versus the HG group.

pendently attenuated this upregulation. These findings suggest that RA suppresses high glucose-induced VEGFA expression at both transcriptional and translational levels.

3. Retinoic acid attenuates high glucose-induced activation of p38 MAPK and nuclear translocation of NF-κB p65

To elucidate the molecular mechanisms underlying RA-mediated suppression of VEGFA, we examined the activation of key inflammatory signaling pathways. ARPE-19 cells were treated with 30 mM high glucose, with or without RA (5 or 20 μM), for 24 hours. Western blot analysis revealed that high glucose markedly increased the phosphorylation of p38 MAPK, which was suppressed by RA in a dose-dependent manner (Figure 3A). Densitometric analysis showed a significant decrease in the ratio of phosphorylated p38 to total p38 in RA-treated groups (Figure 3B). Furthermore, nuclear fractions were analyzed to assess the translocation of NF-κB p65. High glucose significantly promoted p65 nuclear accumulation, whereas

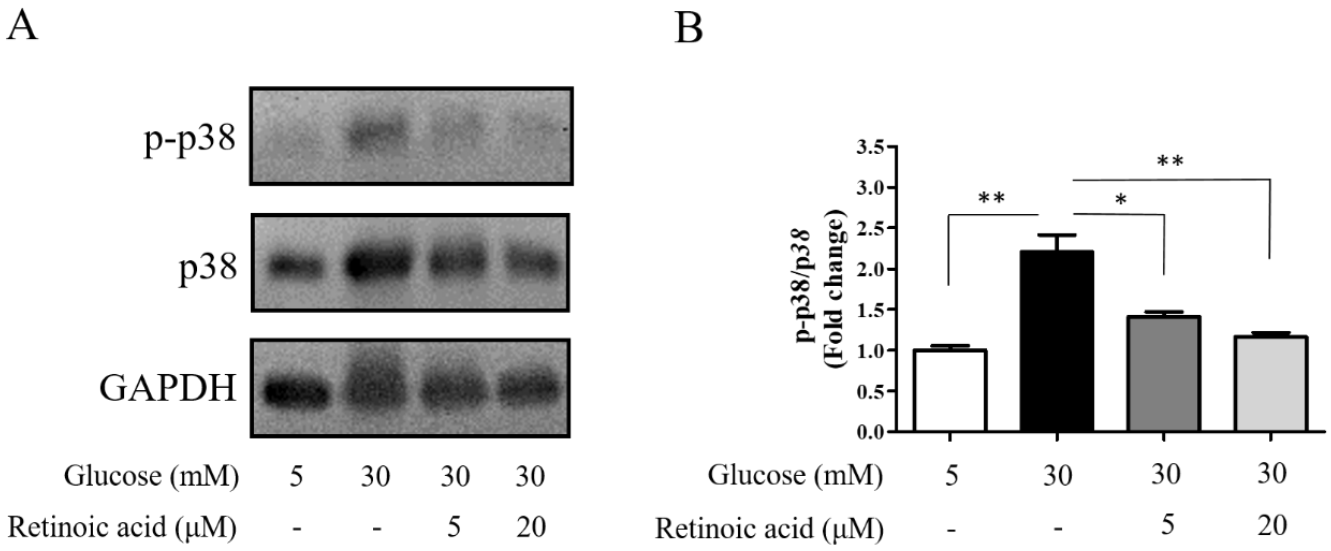

Figure 3. RA suppresses high glucose-induced phosphorylation of p38 MAPK in ARPE-19 cells.
ARPE-19 cells were treated with 30 mM high glucose alone or in combination with RA (5 or 20 μM) for 24 hours. (A) Total protein was extracted and the expression of phosphorylated p38 and total p38 was analyzed by Western blot. (B) Densitometric quantification of p-p38 levels was normalized to total p38, and the relative fold change is presented. Data are shown as mean ± SD from three independent experiments. Statistical significance was determined using Student's *t*-test. $P < 0.05$, $P < 0.01$ versus the HG group.

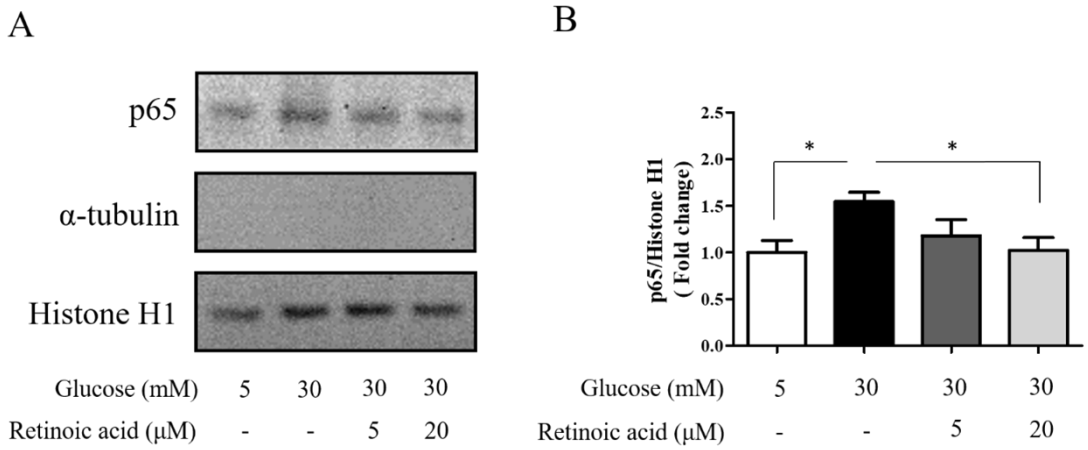

Figure 4. RA inhibits high glucose-induced nuclear translocation of p65 in ARPE-19 cells.
ARPE-19 cells were treated with 30 mM high glucose in the presence or absence of RA (5 or 20 μM) for 24 hours. (A) Nuclear proteins were extracted and subjected to Western blot analysis to assess the nuclear translocation of NF-κB p65. Histone H1 was used as a nuclear loading control. (B) Densitometric quantification of nuclear p65 levels was normalized to histone H1. Data are presented as mean ± SD from three independent experiments. Statistical significance was determined using Student's *t*-test. $P < 0.05$, $P < 0.01$ versus the HG group.

co-treatment with RA attenuated this effect (Figure 4A). Quantification normalized to histone H1 confirmed the RA-induced reduction in nuclear p65 expression (Figure 4B). Together, these results suggest that RA inhibits high glucose-induced VEGFA expression, at least in part, through suppression of p38 MAPK activation and NF-κB nuclear translocation.

Discussion

DR is one of the most common microvascular complications

of diabetes and remains a leading cause of blindness in working-age populations [1]. Hyperglycemia-induced oxidative stress and chronic inflammation are critical contributors to DR pathogenesis, which is characterized by retinal vascular leakage, neovascularization, and the overexpression of pro-angiogenic factors such as VEGFA [21]. In this study, we demonstrated that RA significantly attenuates high glucose-induced VEGFA expression in ARPE-19 cells, and this effect is associated with suppression of p38 MAPK phosphorylation and NF-κB p65 nuclear translocation.

RA is a biologically active derivative of vitamin A that plays a crucial role in regulating cell proliferation, differentiation, and inflammation [18]. Previous studies have reported both pro- and anti-angiogenic effects of RA depending on the cellular context and microenvironment [22,23]. For instance, Tokarz et al. demonstrated that RA modulates VEGFA expression in ARPE-19 cells under oxidative stress [20]. Additionally, Heimsath et al. reported that RA reduces VEGF secretion in ARPE-19 cells grown in hyperglycemic media but not in euglycemic media, suggesting a specific action under stress conditions [24]. However, the specific molecular mechanisms linking RA to the suppression of high glucose-induced VEGFA via the p38 MAPK and NF-κB axis have not been fully elucidated. Our findings provide new evidence supporting the anti-angiogenic potential of RA under diabetic conditions. In the current model, RA treatment reduced both mRNA and protein levels of VEGFA in ARPE-19 cells exposed to high glucose, suggesting a transcriptional level of regulation [25,26].

Mechanistically, our data indicate that RA inhibits the activation of p38 MAPK and the nuclear translocation of NF-κB p65 in response to high glucose stimulation. The p38 MAPK pathway is a well-established mediator of stress and inflammatory responses, and its activation has been implicated in the upregulation of VEGFA and the progression of DR [12,13]. Likewise, NF-κB is a central transcription factor that controls the expression of numerous pro-inflammatory and angiogenic genes. The observed suppression of these pathways by RA suggests that its protective effect may be mediated through inhibition of oxidative stress-responsive signaling cascades. Notably, the inhibitory effect of RA was dose-dependent and paralleled its ability to reduce VEGFA expression.

Interestingly, our CCK-8 assay results also revealed that RA at higher concentrations (>40 μM) significantly reduced cell viability. This observation underscores a critical boundary for clinical application: while low doses (5–20 μM) are protective, exceeding this therapeutic window poses a risk of retinal toxicity. Thus, precise dosage control is essential to balance efficacy against potential cytotoxic effects. It remains to be determined whether RA exerts similar anti-inflammatory and anti-angiogenic effects in vivo, and whether additional mechanisms such as modulation of reactive oxygen species or epigenetic regulation may contribute to its action.

It is important to acknowledge that the in vivo environment of the diabetic retina is far more complex than the in vitro model used in this study [27]. In diabetic patients, enhanced blood plasma glucose significantly alters transcellular pathways. The retinal pigment epithelium relies on gradient-dependent inflow of glucose via membrane transporters (such as GLUT1) to travel across the blood-retinal barrier [28]. Chronic hyperglycemia may lead to the downregulation or saturation of these transmembrane channels, a phenomenon that involves intricate osmotic and metabolic regulations not fully captured in a static high-glucose culture system [29]. Therefore, while our results demonstrate the efficacy of RA intracellularly, future in vivo studies must consider these transport dynamics to validate the therapeutic potential fully.

RA offers several advantages: it is a naturally derived metabolite of vitamin A with a well-documented pharmacological profile [18,30]. In our study, RA at 5–20 μM effectively suppressed high glucose-induced VEGFA upregulation in ARPE-19 cells without cytotoxicity, whereas only much higher doses (>40 μM) impaired cell viability – indicating a favorable therapeutic window for retinal cells [20]. By contrast, standard anti-VEGF treatment requires frequent intravitreal injections that are invasive and costly, with cumulative risks of complications such as endophthalmitis, hemorrhage, or retinal detachment as the number of injections increases [8]. Moreover, anti-VEGF agents target a single angiogenic factor, whereas RA modulates upstream inflammatory and oxidative stress pathways in addition to downregulating VEGF, potentially providing a broader, more holistic protective effect on the diabetic retina. These advantages highlight RA as a low-toxicity, non-prescription therapeutic candidate that could complement or reduce the need for conventional anti-VEGF therapy in DR, pending further in vivo validation.

Overall, our findings suggest that RA could serve as a promising candidate for the modulation of hyperglycemia-induced retinal inflammation and angiogenesis, potentially offering new insight into adjunctive treatment strategies for early-stage diabetic retinopathy.

Limitations

The present study has several limitations that should be acknowledged. First, a limitation of the current study is the reliance on pharmacological treatment to infer the signaling mechanisms. While our results indicate a strong association between RA treatment, the suppression of p38 MAPK/NF-κB signaling, and the downregulation of VEGFA, we did not employ specific rescue assays, gene knockdown (siRNA), or overexpression systems to conclusively validate the causal relationship. The present study was designed as a proof-of-concept to identify the therapeutic potential and the therapeutic window of RA in a high-glucose environment; thus, future studies utilizing these genetic tools are necessary to fully dissect the molecular hierarchy. Second, this study was conducted exclusively in ARPE-19 cells. While this is a widely accepted model for RPE research, it does not capture the complex multicellular interactions of the retinal neurovascular unit. Third, it is important to note that VEGFA is not solely a pathological factor; it is also a vital cy-

tokine secreted by innate immune cells, such as macrophages, neutrophils, and mast cells, for physiological maintenance [31]. Therefore, dose optimization of RA will be a crucial practice in clinical translation to ensure that the treatment targets pathological neovascularization without suppressing the beneficial, physiological functions of this cytokine.Regarding the clinical relevance of the dosage, we acknowledge that the concentrations used in this study (5–20 μM) are higher than the peak plasma concentrations (<2 μM) observed in patients treated with oral ATRA (Vesanoid) for APL [30]. However, in vitro models often require higher concentrations to elicit responses due to short exposure times and drug binding to serum proteins in the culture medium. More importantly, for the treatment of diabetic retinopathy, we propose local administration (e.g., intravitreal injection or sustained-release implants) rather than systemic oral dosing. Local delivery could allow achievable therapeutic concentrations within the retina while minimizing systemic exposure and the associated adverse effects highlighted in the FDA label for oral ATRA [8,30].

Conclusion

In summary, this study demonstrates that all-trans retinoic acid (RA) effectively suppresses high glucose-induced VEGFA expression in ARPE-19 cells, potentially through inhibition of the p38 MAPK and NF-κB signaling pathways. These findings highlight the anti-inflammatory and anti-angiogenic properties of RA in retinal pigment epithelial cells under hyperglycemic stress and suggest its potential utility in the prevention or treatment of diabetic retinopathy. Further studies, including in vivo validation and exploration of RA-mediated transcriptional regulation, are warranted to clarify its therapeutic application in retinal diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data availability

The authors declare that the data are available upon reasonable request.

Ethical Approval

Not applicable.

References

1. G. Zhang, W. Chen, H. Chen, J. Lin, L.P. Cen, P. Xie, Y. Zheng, T.K. Ng, M.E. Brelén, M. Zhang, C.P. Pang, Risk factors for diabetic retinopathy, diabetic macular edema, and sight-threatening diabetic retinopathy, Asia-Pacific Journal of Ophthalmology 13 (2024). https://doi.org/10.1016/j.apjo.2024.100067.
2. W. Wang, A.C.Y. Lo, Diabetic retinopathy: Pathophysiology and treatments, Int J Mol Sci 19 (2018). https://doi.org/10.3390/ijms19061816.
3. E.J. Duh, J.K. Sun, A.W. Stitt, Diabetic retinopathy: Current understanding, mechanisms, and treatment strategies, JCI Insight 2 (2017). https://doi.org/10.1172/JCI.INSIGHT.93751.
4. M. Whitehead, S. Wickremasinghe, A. Osborne, P. Van Wijngaarden, K.R. Martin, Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies, Expert Opin Biol Ther 18 (2018) 1257–1270. https://doi.org/10.1080/14712598.2018.1545836.
5. S. Dragoni, P. Turowski, Polarised VEGFA signalling at vascular blood–neural barriers, Int J Mol Sci 19 (2018). https://doi.org/10.3390/ijms19051378.
6. H. Funatsu, H. Noma, T. Mimura, S. Eguchi, S. Hori, Association of Vitreous Inflammatory Factors with Diabetic Macular Edema, Ophthalmology 116 (2009) 73–79. https://doi.org/10.1016/j.ophtha.2008.09.037.
7. K. Itoh, M. Furuhashi, Y. Ida, H. Ohguro, M. Watanabe, S. Suzuki, F. Hikage, Detection of significantly high vitreous concentrations of fatty acid-binding protein 4 in patients with proliferative diabetic retinopathy, Sci Rep 11 (2021). https://doi.org/10.1038/s41598-021-91857-1.
8. K.G. Falavarjani, Q.D. Nguyen, Adverse events and complications associated with intravitreal injection of anti-VEGF agents: A review of literature, Eye (Basingstoke) 27 (2013) 787–794. https://doi.org/10.1038/eye.2013.107.
9. T. Yamamoto, A. Kanda, S. Kase, S. Ishida, Hypoxia Induces Galectin-1 Expression Via Autoinduction of Placental Growth Factor in Retinal Pigment Epithelium Cells, Invest Ophthalmol Vis Sci 62 (2021). https://doi.org/10.1167/IOVS.62.2.22.
10. A. Klettner, J. Roider, Constitutive and oxidative-stress-induced expression of VEGF in the RPE are differently regulated by different Mitogen-activated protein kinases, Graefe's Archive for Clinical and Experimental Ophthalmology 247 (2009) 1487–1492. https://doi.org/10.1007/s00417-009-1139-x.
11. C.N. Nagineni, V.K. Kommineni, A. William, B. Detrick, J.J. Hooks, Regulation of VEGF expression in human retinal cells by cytokines: Implications for the role of inflammation in age-related macular degeneration, J Cell Physiol 227 (2012) 116–126. https://doi.org/10.1002/jcp.22708.
12. M.C. Marazita, A. Dugour, M.D. Marquioni-Ramella, J.M. Figueroa, A.M. Suburo, Oxidative stress-induced premature senescence dysregulates VEGF and CFH expression in retinal pigment epithelial cells: Implications for Age-related Macular Degeneration, Redox Biol 7 (2016) 78–87. https://doi.org/10.1016/j.redox.2015.11.011.
13. G. Maugeri, C. Bucolo, F. Drago, S. Rossi, M. Di Rosa, R. Imbesi, V. D'Agata, S. Giunta, Attenuation of High Glucose-Induced Damage in RPE Cells through p38 MAPK Signaling Pathway Inhibition, Front Pharmacol 12 (2021). https://doi.org/10.3389/fphar.2021.684680.

14. D. Qin, Y.R. Jiang, Tangeretin Inhibition of High-Glucose-Induced IL-1 β, IL-6, TGF- β 1, and VEGF Expression in Human RPE Cells, J Diabetes Res 2020 (2020). https://doi.org/10.1155/2020/9490642.
15. S. Devaraj, S.K. Venugopal, U. Singh, I. Jialal, I. Jialal, Hyperglycemia Induces Monocytic Release of Interleukin-6 via Induction of Protein Kinase C-and, 2005. http://diabetesjournals.org/diabetes/article-pdf/54/1/85/650530/zdb00105000085.pdf.
16. W. Li, Q. Xing, Z. Liu, R. Liu, Y. Hu, Q. Yan, X. Liu, J. Zhang, The signaling pathways of traditional Chinese medicine in treating diabetic retinopathy, Front Pharmacol 14 (2023). https://doi.org/10.3389/fphar.2023.1165649.
17. T.T. Schug, D.C. Berry, N.S. Shaw, S.N. Travis, N. Noy, Opposing Effects of Retinoic Acid on Cell Growth Result from Alternate Activation of Two Different Nuclear Receptors, Cell 129 (2007) 723–733. https://doi.org/10.1016/j.cell.2007.02.050.
18. N.B. Ghyselinck, G. Duester, Retinoic acid signaling pathways, Development (Cambridge) 146 (2019). https://doi.org/10.1242/dev.167502.
19. A. Dutta, T. Sen, A. Chatterjee, All-trans retinoic acid (ATRA) downregulates MMP-9 by modulating its regulatory molecules, Cell Adh Migr 4 (2010) 409–418. https://doi.org/10.4161/cam.4.3.11682.
20. P. Tokarz, A.W. Piastowska-Ciesielska, K. Kaarniranta, J. Blasiak, All-trans retinoic acid modulates DNA damage response and the expression of the VEGF-A and MKI67 genes in ARPE-19 cells subjected to oxidative stress, Int J Mol Sci 17 (2016). https://doi.org/10.3390/ijms17060898.
21. H. Rao, J.A. Jalali, T.P. Johnston, P. Koulen, Emerging Roles of Dyslipidemia and Hyperglycemia in Diabetic Retinopathy: Molecular Mechanisms and Clinical Perspectives, Front Endocrinol (Lausanne) 12 (2021). https://doi.org/10.3389/fendo.2021.620045.
22. B. Pawlikowski, J. Wragge, J.A. Siegenthaler, Retinoic acid signaling in vascular development, Genesis 57 (2019). https://doi.org/10.1002/dvg.23287.
23. A. Saito, A. Sugawara, A. Uruno, M. Kudo, H. Kagechika, Y. Sato, Y. Owada, H. Kondo, M. Sato, M. Kurabayashi, M. Imaizumi, S. Tsuchiya, S. Ito, All-trans retinoic acid induces in vitro angiogenesis via retinoic acid receptor: Possible involvement of paracrine effects of endogenous vascular endothelial growth factor signaling, Endocrinology 148 (2007) 1412–1423. https://doi.org/10.1210/en.2006-0900.
24. E.G. Heimsath, R. Unda, E. Vidro, A. Muniz, E.T. Villazana-Espinoza, A. Tsin, ARPE-19 cell growth and cell functions in euglycemic culture media, Curr Eye Res 31 (2006) 1073–1080. https://doi.org/10.1080/02713680601052320.
25. B. Rbara, V. Diaz, M.-C.C. Lenoir, A. Ladoux, C. Frelin, M. Dé Marchez, S. Michel, Regulation of Vascular Endothelial Growth Factor Expression in Human Keratinocytes by Retinoids*, 2000. http://www.jbc.org.
26. E. Karkeni, L. Bonnet, J. Astier, C. Couturier, J. Dalifard, F. Tourniaire, J.F. Landrier, All-trans-retinoic acid represses chemokine expression in adipocytes and adipose tissue by inhibiting NF-κB signaling, Journal of Nutritional Biochemistry 42 (2017) 101–107. https://doi.org/10.1016/j.jnutbio.2017.01.004.
27. T. Yumnamcha, M. Guerra, L.P. Singh, A.S. Ibrahim, Metabolic dysregulation and neurovascular dysfunction in diabetic retinopathy, Antioxidants 9 (2020) 1–22. https://doi.org/10.3390/antiox9121244.
28. A. Swarup, I.S. Samuels, B.A. Bell, J.Y. S Han, J. Du, E. Massenzio, E. Dale Abel, K. Boesze-Battaglia, N.S. Peachey, N.J. Philp, Modulating GLUT1 expression in retinal pigment epithelium decreases glucose levels in the retina: impact on photoreceptors and Müller glial cells, Am J Physiol Cell Physiol 316 (2019) 121–133. https://doi.org/10.1152/ajpcell.00410.2018.-The.
29. P. González, P. Lozano, G. Ros, F. Solano, Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections, Int J Mol Sci 24 (2023). https://doi.org/10.3390/ijms24119352.
30. J. Jing, C. Nelson, J. Paik, Y. Shirasaka, J.K. Amory, N. Isoherranen, Physiologically Based Pharmacokinetic Model of All-trans-Retinoic Acid with Application to Cancer Populations and Drug Interactions, Journal of Pharmacology and Experimental Therapeutics 361 (2017) 246–258. https://doi.org/10.1124/jpet.117.240523.
31. C. Murdoch, M. Muthana, S.B. Coffelt, C.E. Lewis, The role of myeloid cells in the promotion of tumour angiogenesis, Nat Rev Cancer 8 (2008) 618–631. https://doi.org/10.1038/nrc2444.