MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 6946-6954
DOI: 10.12659/MSM.917186

Received: 2019.04.25
Accepted: 2019.05.07
Published: 2019.09.16

The Effects of the CXCR4 Antagonist, AMD3465, on Human Retinal Vascular Endothelial Cells (hRVECs) in a High Glucose Model of Diabetic Retinopathy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Funds Collection G

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Source of support: Departmental sources

Background: High blood glucose levels in diabetes result in retinal angiogenesis, which is the key feature of diabetic retinopathy. This study aimed to investigate the effects of the CXCR4 antagonist, AMD3465, on human retinal vascular endothelial cells (hRVECs) *in vitro*.

Material/Methods: Cell viability and the protein expression levels of CXCR4 and stromal cell-derived factor 1 (SDF-1) were evaluated in high glucose (HG)-treated human retinal vascular endothelial cells (hRVECs). The cell counting kit 8 (CCK-8) assay, the colony formation assay, immunofluorescence, and Western blot were used to investigate the effects of AMD3465 on hRVEC cell viability, colony formation, cell proliferation, and expression of CXCR4 and SDF-1. Cell apoptosis and angiogenesis were assessed by flow cytometry and Western blot.

Results: Treatment with high glucose reduced the viability of hRVECs and increased the protein expression levels of CXCR4 and SDF-1. Following treatment with AMD3465, the colony formation capacity and cell proliferation in hRVECs increased, and there was a significant reduction in apoptosis rate compared with the untreated cells. AMD3465 significantly reduced the expression of angiogenesis-associated proteins, including ICAM1, VCAM1, VEGF, and AngII. AMD3465 significantly reduced the protein expression levels of TNF-α, IL-1β, NF-κB, and p-p65.

Conclusions: The CXCR4 antagonist, AMD3465, reduced apoptosis of HG-treated hRVECs in an *in vitro* model of diabetic retinopathy.

MeSH Keywords: Diabetic Retinopathy • Protective Agents • Receptors, CXCR4

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/917186

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Background

Worldwide, diabetes mellitus is a common chronic disease [1]. Type 2 diabetes mellitus (T2DM) is associated with complications that include long-term damage to vital organs, including the eyes, kidneys, nerves, and blood vessels [2]. Diabetic retinopathy is associated with chronic diabetes mellitus and is the main ocular complication of the disease [3,4]. Previous studies have shown that diabetic retinopathy can be caused by biochemical changes, apoptosis, inflammation, oxidative stress, and angiogenesis [5–7]. Among these factors, angiogenesis is one of the most detrimental events responsible for the progression of diabetic retinopathy. The formation of new blood vessels from the existing vasculature generates anatomical and physiological changes that can result in blindness [8].

CXCR4 is a chemokine receptor that selectively binds to the CXC chemokine stromal cell-derived factor 1 (SDF-1), or CXCL12 [9]. CXCR4 is associated with chemotaxis, invasion, angiogenesis, and cell proliferation [10]. The SDF-1/CXCR4 axis takes part in many physiological processes, including immune cells homeostasis and cell trafficking [11]. This axis contributes to the activation of tip cells and microglia in retinal angiogenesis [12]. Also, the expression of CXCR4 participates in angiogenesis associated with increased expression of SDF-1 in injured tissues [13]. Because CXCR4 is a target for several diseases, there is therapeutic potential for novel CXCR4 antagonists. AMD3465 is a CXCR4 antagonist that has been shown to inhibit tumor cell growth in a mouse model by the downregulation of glycogen synthase kinase-3 (GSK3) and decreased angiogenesis [14]. GSK3 is an important factor involved in the signaling pathways to regulate angiogenesis [15]. Also, another CXCR4 antagonist, AMD3100, has been shown to reduce the expression of different angiogenic factors and to cause a significant reduction in tumor growth and invasion [16,17]. However, although there is considerable knowledge concerning the function of the CXCR4 antagonist, AMD3465, in tumor angiogenesis, few studies have identified its relationship with diabetic retinopathy. Therefore, this study aimed to investigate the effects of the CXCR4 antagonist, AMD3465, on human retinal vascular endothelial cells (hRVECs) treated with high levels of glucose.

Material and Methods

Cell culture and establishment of a high glucose (HG)-treated human retinal vascular endothelial cell (hRVEC) model of diabetic retinopathy

Human retinal vascular endothelial cells (hRVECs) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA), 100 U/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA), and 0.1 mg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in 5% CO_2. The normal group (cells cultured with 5.5 mmol/L glucose), the low group (cells cultured in 20 mmol/L glucose), the middle group (cells cultured in 80 mmol/L glucose), and the high group (cells cultured in 120 mmol/L glucose) were studied to determine the optimum concentration of glucose to be used in the following experiments. The hRVECs were divided into three groups: the control group (cells cultured with 5.5 mmol/L glucose), the model group (cells cultured in 80 mmol/L glucose), and the model+AMD3465 group (cells cultured in 80 mmol/L glucose and 2 μM of AMD3465).

Cell viability assay

The hRVEC cells were seeded in 96-well plates at 4×10^3 cells per well with glucose at different concentrations and incubated for 72 h. Cell viability was measured using a cell counting kit 8 (CCK-8) assay kit (Dojindo Laboratories, Tokyo, Japan) according to the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate spectrophotometer.

Colony formation assay

The cells were seeded in 12-well plates at 5×10^4 cells/mL with glucose (80 mmol/L) and AMD3465 (2 μM). After incubation for 10 days, the plates were stained with 0.5% crystal violet (Wako, Osaka, Japan) in 4% paraformaldehyde for 5 minutes to count the colony numbers. The cell group containing more than 50 cells was identified as a colony. Each assay was repeated five times.

Immunofluorescence staining

The hRVEC cells were fixed on glass slides with 4% paraformaldehyde for 15 min and incubated in PBS with 0.1% Triton X-100 for 20 min to permeabilize the cells. After blocking using 5% dried skimmed milk powder in 0.01 M PBS, the cells were incubated with an antibody to Ki67 (SAB4501880) (Sigma-Aldrich, St. Louis MO, USA) overnight at 4°C. After washing three times in PBS, the cells were then incubated with Alexa Fluor-conjugated secondary antibodies (Life Technologies, Carlsbad, CA, USA) for 2 h at room temperature. The sections were coverslipped, and the fluorescence staining was detected with an Olympus FV1000 confocal laser scanning microscope (Olympus, Shinjuku, Tokyo, Japan).

Western blot

The hRVEC cells were lysed with ice-cold RIPA buffer. After centrifugation at 12000 rpm for 10 min, the supernatant was collected and the concentration of protein was determined using a BCA protein assay reagent kit (Beyotime, Shanghai, China). The proteins (15 μg) were separated by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene

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fluoride (PVDF) membrane. Then membrane was blocked with 5% dried skimmed milk powder for 2 h on a horizontal shaking table. The PVDF membranes were then incubated overnight at 4°C with the following primary antibodies to: CXCR4 (1: 500) (ab1670; Abcam, Cambridge, MA, USA); SDF-1 (1 µg/ml) (ab18919; Abcam, Cambridge, MA, USA); ICAM1 (1: 1000) (ab20; Abcam, Cambridge, MA, USA); VCAM1 (1: 1000) (ab98954; Abcam, Cambridge, MA, USA); VEGF (1: 2000) (ab53465; Abcam, Cambridge, MA, USA); AngII (1: 1000) (ab236317; Abcam, Cambridge, MA, USA); CDK2 (5 µg/ml) (ab6433; Abcam, Cambridge, MA, USA); cyclin E (1: 2000) (ab71535; Abcam, Cambridge, MA, USA); p21 (1: 3000) (ab227443; Abcam, Cambridge, MA, USA); Bcl2 (1: 500) (ab196495; Abcam, Cambridge, MA, USA); Bax (1: 500) (ab53154; Abcam, Cambridge, MA, USA); TNF-α (1: 2000) (ab6671; Abcam, Cambridge, MA, USA); IL-1β (1: 1000) (ab2105; Abcam, Cambridge, MA, USA); NF-κB (1: 1000) (ab194729; Abcam, Cambridge, MA, USA); p65 (1: 1000) (ab16502; Abcam, Cambridge, MA, USA); p-p65 (1: 1000) (ab28856; Abcam, Cambridge, MA, USA); and GAPDH (1: 2500) (ab9485; Abcam, Cambridge, MA, USA). The membranes were incubated with a horseradish peroxidase (HRP)-labeled secondary antibody (Abcam, Cambridge, MA, USA). The results were visualized with an ECL detection kit. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to quantify the density of the bands.

Flow cytometry

Apoptosis assays were performed using a fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit (BD Biosciences Pharmingen, San Jose, CA, USA). Briefly, after incubation for 48 h, the hRVEC cells were harvested and washed with cold PBS and resuspended at a concentration of 1×10^6 cells/ml. Apoptotic cells were analyzed using flow cytometry. Statistical analysis was performed using Flowjo software (Tree Star, Ashland, OR, USA).

Statistical analysis

Data were analyzed using SPSS version 20.0 software. The results were expressed as the mean ± standard deviation (SD). Student's t-test was used for comparison between the two groups, and one-way analysis of variance (ANOVA) was performed to compare three or more groups. A P-value <0.05 was considered to be statistically significant.

Results

The effect of high glucose on the viability of human retinal vascular endothelial cells (hRVECs)

To determine the appropriate concentration of glucose for this study, we first tested the viability of hRVECs with different concentrations of glucose. Glucose at 5.5 mmol/L was used to simulate the condition of hyperglycemia *in vitro*. The results of the cell counting kit 8 (CCK-8) assay showed that high glucose reduced the viability of hRVECs in a dose-dependent manner (Figure 1).

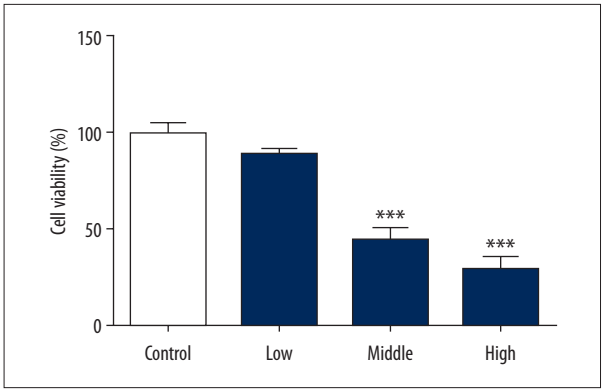

Figure 1. High glucose affected the cell viability of human retinal vascular endothelial cells (hRVECs) in a dose-dependent manner. Data are expressed as the mean ± standard deviation (SD). *** P<0.001 versus control.

The effect of high glucose on protein expression of CXCR4 and SDF-1 in hRVECs

To investigate the effect of high glucose on CXCR4 and SDF-1 in hRVECs, Western blot analysis was performed. As shown in Figure 2, high glucose increased the protein expression of CXCR4 and SDF-1 in a dose-dependent manner when compared with the normal group. Based on this finding, glucose at a concentration of 80 mmol/L was chosen for the subsequent experiments.

The CXCR4 antagonist, AMD3465, promoted the proliferation of high glucose (HG)-treated hRVECs

To determine whether AMD3465 could affect cell proliferation of hRVECs under HG conditions, immunofluorescence staining, the colony formation assay, and Western blot were used. The results showed that the expression of Ki67 was significantly lower in the *in vitro* model than the control. In contrast to the model, the level of Ki67 was increased in hRVECs treated with AMD3465 (Figure 3A). The colony formation assay showed that colony numbers were significantly reduced under high glucose conditions compared with the control, while AMD3465 enhanced the ability of hRVECs to form colonies. Also, there was no significant difference in the colony numbers between the control group and the model+AMD3465 (Figure 3B). Western blot was used to determine the expression of proliferation-associated proteins in HG-treated hRVECs. High glucose levels significantly reduced the expression of CDK2 and cyclin E and increased the expression of p21 compared with

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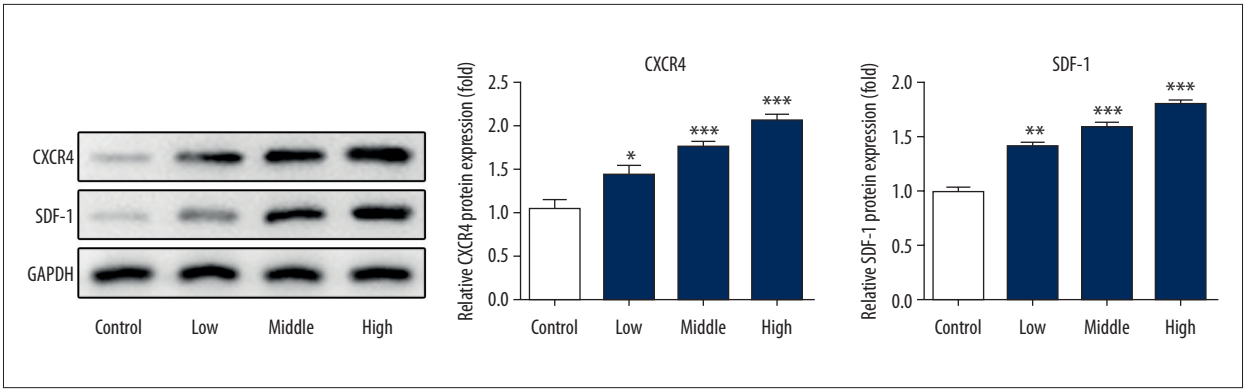
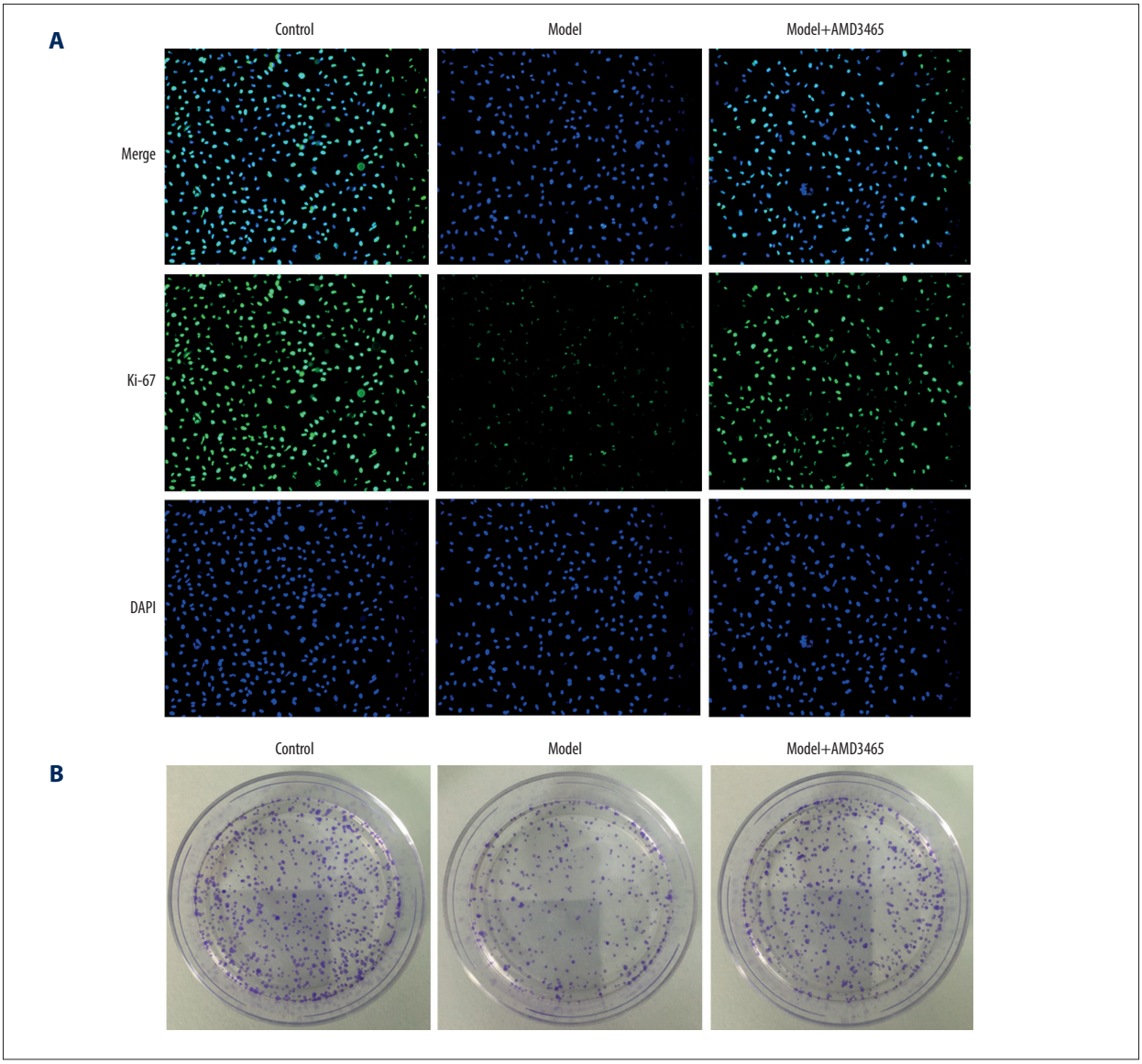


Figure 2. High glucose increased the protein levels of CXCR4 and SDF-1 human retinal vascular endothelial cells (hRVECs) in a dose-dependent manner. Data are expressed as the mean ± standard deviation (SD). * P<0.05, ** P<0.01, *** P<0.001 versus control.



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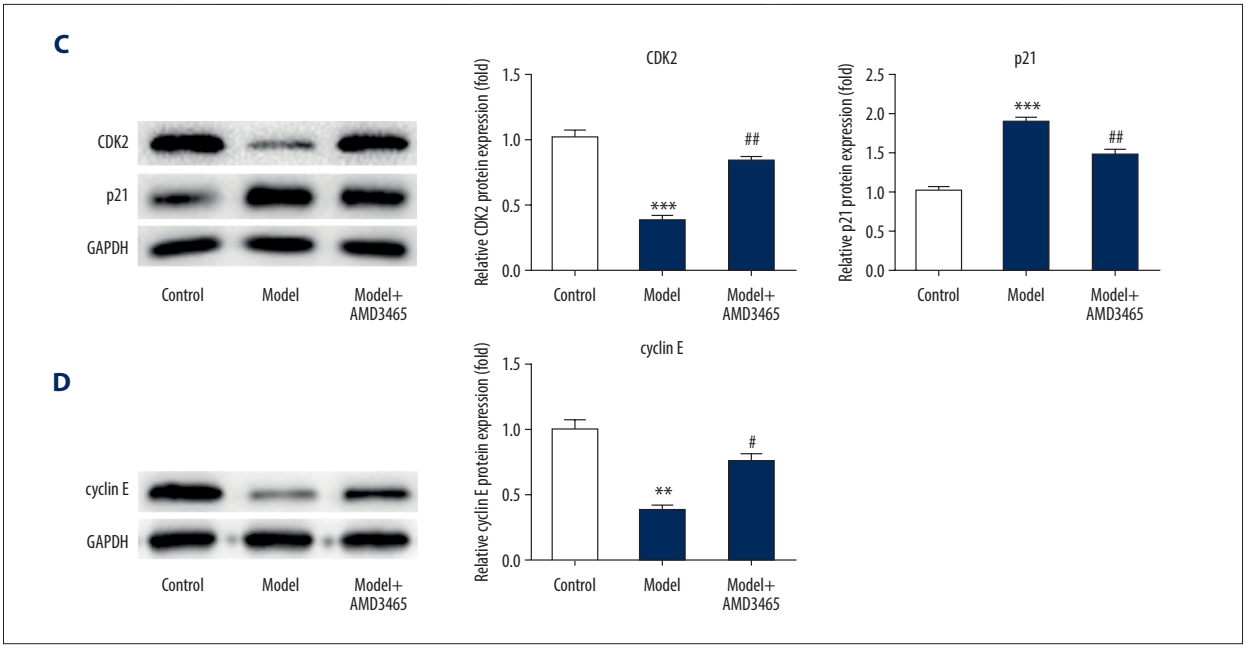

Figure 3. The CXCR4 antagonist, AMD3465, promoted the proliferation of high glucose (HG)-treated human retinal vascular endothelial cells (hRVECs). (**A**) The expression of Ki67 in HG-induced hRVECs was measured by immunofluorescence staining. (**B**) The effects of AMD3465 the growth of HG-cultured hRVECs were determined by the colony formation assay. (**C**) The expression of proliferation-associated proteins, CDK2, p21, and cyclin E were detected by Western blot. Data are expressed as the mean ± standard deviation (SD). ** P < 0.01, *** P<0.001 versus control; # P<0.05, ## P<0.01 versus the model.

the control, while AMD3465 treatment had the opposite results (Figure 3C). These results indicated that AMD3465 facilitated the proliferation of HG-treated hRVECs.

AMD3465 inhibited the apoptosis of HG-treated hRVECs

Flow cytometry and Western blot were used to evaluate the effect of AMD3465 on apoptosis of HG-treated hRVECs. The results showed that high glucose significantly increased the apoptosis rate of HG-treated hRVECs compared with the control. However, AMD3465 treatment significantly reduced cell apoptosis (Figure 4A, 4B). The expression of the pro-apoptotic protein Bax was found to be increased, and the anti-apoptotic protein Bcl-2 was reduced in hRVECs with high glucose treatment, while AMD3465 treatment showed the opposite results (Figure 4C). These results showed that AMD3465 exerted an inhibitory effect on the apoptosis of HG-treated hRVECs.

The effect of AMD3465 on endothelial cell function and angiogenesis in HG-treated hRVECs

To determine whether AMD3465 affected endothelial cell function and angiogenesis, cell adhesion molecules and angiogenesis-related proteins were studied. The results of Western blot analysis showed that the protein levels of ICAM1, VCAM1, VEGF, and AngII in HG-treated hRVECs were significantly increased. The protein levels were decreased in HG-treated hRVECs when also treated with AMD3465 (Figure 5). These results showed that AMD3465 improved endothelial cell function, but inhibited angiogenesis in HG-treated hRVECs.

AMD3465 protected HG-treated hRVECs by inhibiting the NF-κB signaling pathway

To explore the potential mechanism of AMD3465 in protecting HG-treated hRVECs, we detected the NF-κB signaling pathway by Western blot analysis. The results showed that the expression of TNF-α, IL-1β, NF-κB, and p-p65 were significantly increased in HG-cultured hRVECs compared with the control. AMD3465 treatment reduced the expression of TNF-α, IL-1β, NF-κB, and p-p65. The protein expression of p65 was unchanged in the three groups (Figure 6). These data showed that AMD3465 protected HG-treated hRVECs partly by inhibiting the NF-κB signaling pathway.

Discussion

The findings from the present study of an *in vitro* model of diabetic retinopathy that used high glucose (HG)-treated human retinal vascular endothelial cells (hRVECs), showed that the CXCR4 antagonist, AMD3465, promoted endothelial cell proliferation, improved endothelial cell function, and inhibited apoptosis and angiogenesis. These findings indicated that

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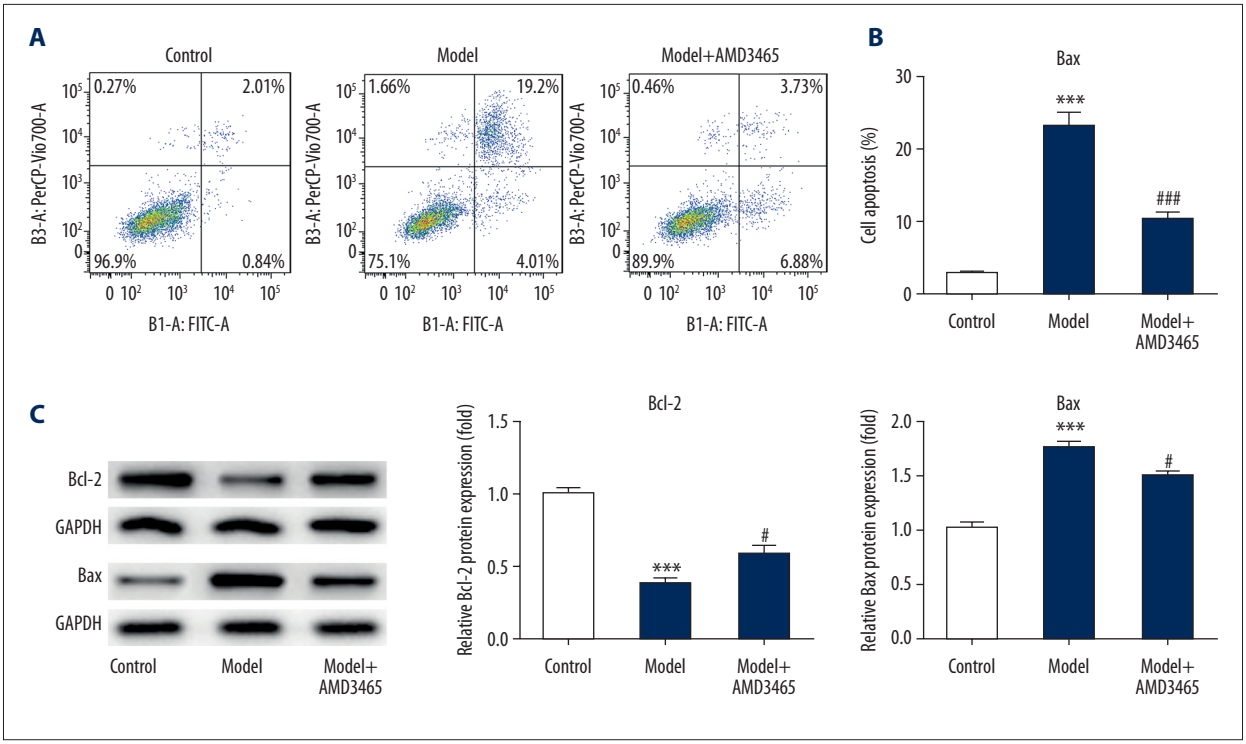

Figure 4. The CXCR4 antagonist, AMD3465, inhibited apoptosis induced by high glucose in human retinal vascular endothelial cells (hRVECs). (**A**) Flow cytometry evaluated cell apoptosis in high glucose (HG)-treated hRVECs. (**B**) Western blot was used to examine the levels of apoptosis-related proteins, Bax and Bcl-2. Data are expressed as the mean ± standard deviation (SD). *** P<0.001 versus control; # P<0.05, ### P<0.001 versus the model.

AMD3465 protected hRVECs from the effects of high glucose by inhibiting the NF-κB signaling pathway.

High glucose can disrupt the physiology of normal human endothelial cells [18–21]. High glucose induces hyperosmolarity, which promotes angiogenesis and diabetic retinopathy [22,23]. Also, hypoxia induced by high glucose in retinal tissues stimulates angiogenesis by modulating the balance of pro-angiogenic and anti-angiogenic mediators, leading to retinal neovascularization [24–26]. Retinal neovascularization is the hallmark of diabetic retinopathy [27]. The SDF-1α/CXCR4 signaling axis is associated with many of these endogenous processes and has a critical role in promoting angiogenesis [28]. A previous study showed that the SDF-1/CXCR4 axis contributed to vasculogenesis and the migration of inflammatory cells by the recruitment of bone marrow-derived endothelial progenitor cells (EPCs) [29]. Another study showed that Danshensu promoted angiogenesis after myocardial infarction in rats and promoted the function of EPCs by the SDF-1α/CXCR4 axis [30]. Overexpression of CXCR4 in mesenchymal stem cells promoted migration, neuroprotection, and angiogenesis in a rat cerebral ischemia model [31]. Our results showed that high glucose reduced cell viability and increased the protein expression of CXCR4 in hRVECs, which is consistent with previous reports.

Due to the over-activation of the SDF-1/CXCR4 axis induced by high glucose, a previous study showed that VEGF and EGFR levels were increased, which resulted in angiogenesis and apoptosis [32]. AMD3465 is a specific antagonist of CXCL12/CXCR4-mediated interactions, including ligand binding and receptor activation [33]. CXCR4 blockade has been shown to result in eosinophil recruitment in granuloma formation and to inhibit immune activation of T-cell in lymphoid tissue [34]. Recent studies have shown that AMD3465 inhibited breast cancer cell migration *in vitro* and prevented breast cancer cell growth and metastasis *in vivo* [14]. CXCR4 antagonists have been shown to significantly inhibit the proliferation, migration, and invasion of SW780 cells [35]. Our results indicated that AMD3465 promoted cell proliferation and inhibited cell apoptosis in HG-treated hRVECs, which is inconsistent with previous reports. We speculated that AMD3465 might regulate the proliferation of normal cells and malignant cells through different signaling pathways, but further studies should be performed to confirm this. Also, AMD3465 has previously been shown to inhibit angiogenesis in breast cancer by abolishing the phosphorylation of glycogen synthase kinase-3 (GSK3), which promotes angiogenesis and tumor progression [14]. Another CXCR4 antagonist, AMD3100, prevented the development of HPS via suppressing pulmonary angiogenesis in CBDL rats and reducing pulmonary C-Kit-positive cells [36]. Our results showed that

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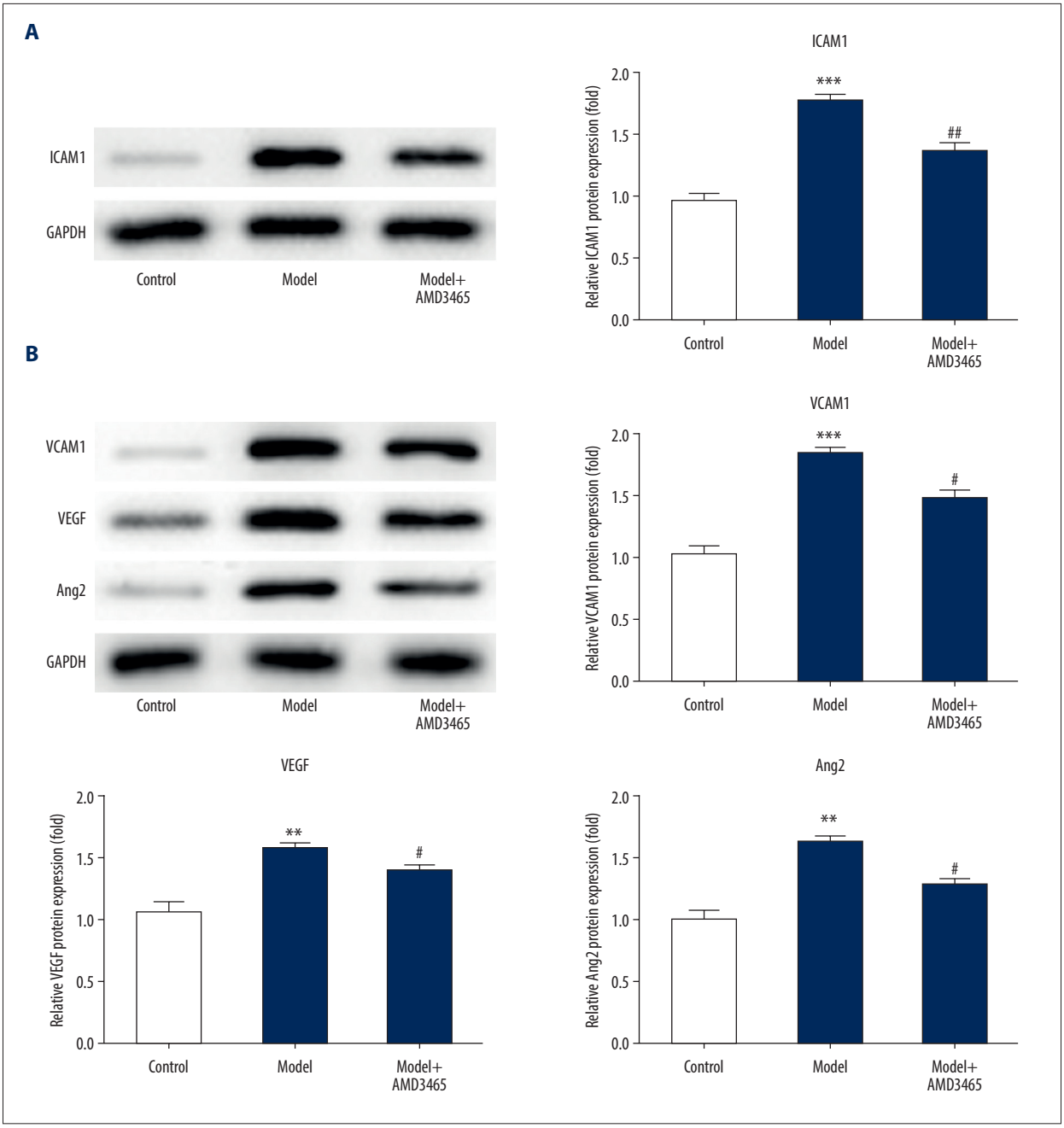

Figure 5. The CXCR4 antagonist, AMD3465, improved endothelial cell function and inhibited angiogenesis induced by high glucose in human retinal vascular endothelial cells (hRVECs). The protein expression levels of ICAM1, VCAM1, VEGF, and AngII in HG-induced hRVECs with AMD3465 treatment were assessed by Western blot. GAPDH was used as an internal reference. Data are expressed as the mean ± standard deviation (SD). ** P<0.01, *** P<0.001 versus control; # P<0.05, ## P<0.01 versus the model.

AMD3465 significantly reduced the expression of the angiogenesis-related proteins, VEGF, and AngII, in high glucose (HG)-treated hRVECs. These findings suggested that AMD3465 protected HG-exposed hRVECs by regulating cell proliferation, apoptosis, and angiogenesis.

Previous studies have shown that diabetes mellitus could disrupt the pathways that regulate the physiology of NF-κB, and that high glucose can activate several signaling pathways, including JNK, P38, MAPK, and NF-κB. Several pathological changes induced by high glucose lead to overactivation of NF-κB, such as accumulation of advanced glycation end products and

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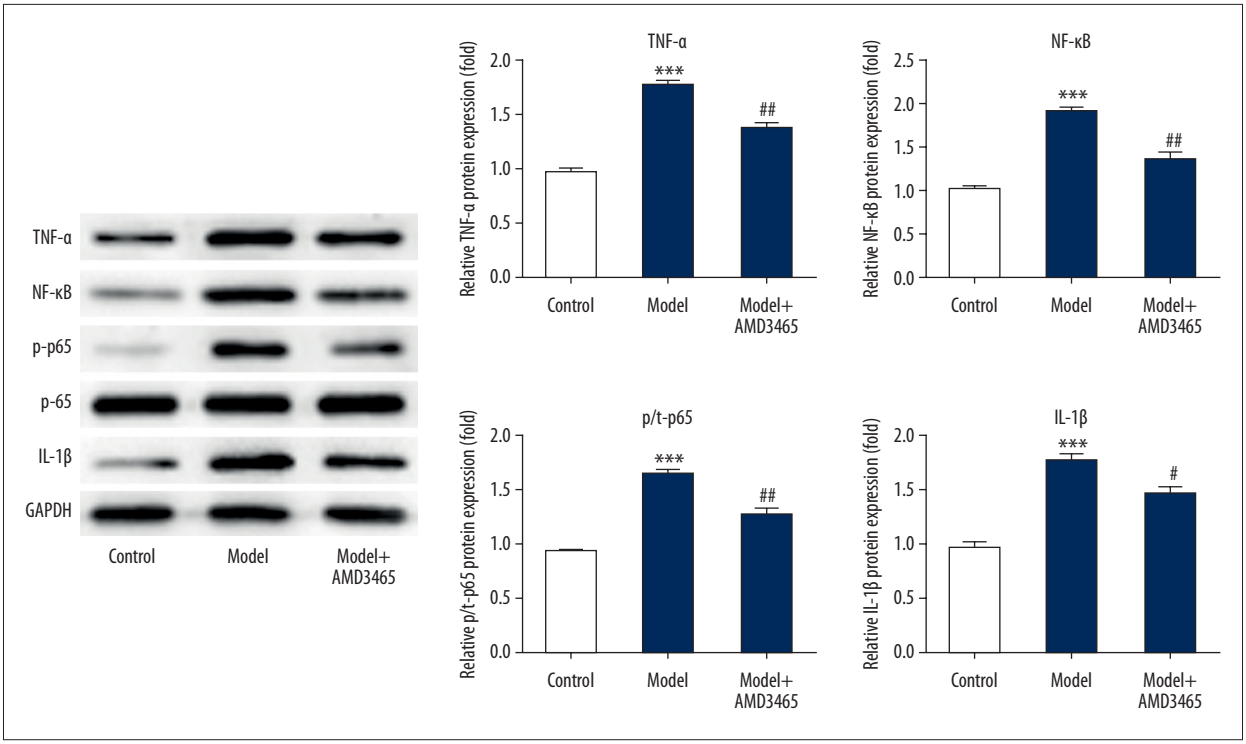

Figure 6. The CXCR4 antagonist, AMD3465, exerted its effects in high glucose (HG)-treated human retinal vascular endothelial cells (hRVECs) by regulating NF-κB activation. Western blot, using specific antibodies, studied the effects of AMD3465 on the protein levels of TNF-α, IL-1β, NF-κB, and p-p65 in the NF-κB signaling pathway. GAPDH was used as an internal reference. Data are expressed as the mean ± standard deviation (SD). *** P<0.001 versus control; # P<0.05, ## P<0.01 versus the model.

oxidative stress [8,21]. This overactivation is responsible for the progression of angiogenesis during diabetic retinopathy. Therefore, we speculated that the NF-κB signal pathway plays critical roles in the progression of diabetic retinopathy. In recent studies, diabetic retinopathy could be prevented by inhibiting the activation of NF-κB in a rat model of HG-induced diabetic retinopathy treated with naringin [37], resolvin D1 [38], and resveratrol [39]. In the present study, the findings showed that AMD3465 treatment effectively inhibited the activation of NF-κB in HG-treated hRVECs to protect retinal endothelial cells from apoptosis and angiogenesis, but the underlying mechanisms remain to be determined.

Conclusions

This study aimed to investigate the effects of the CXCR4 antagonist, AMD3465, on human retinal vascular endothelial cells (hRVECs) *in vitro*. The findings from this study showed that treatment with AMD3465 could inhibit high glucose (HG)-induced apoptosis by reducing the level of Bax and Bcl-2 as well as promoting the proliferation of HG-induced hRVECs. AMD3465 suppressed HG-induced angiogenesis by reducing the activity of VEGF and AngII and inhibited the NF-κB signaling pathway. These findings indicated that AMD3465 might have therapeutic potential in the prevention of the progression of diabetic retinopathy.

Competing interests

None.

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References:

1. Ogurtsova K, Da Rocha Fernandes JD, Huang Y et al: IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract, 2017; 128: 40–50
2. Hinchliffe RJ, Brownrigg JR, Andros G et al: Effectiveness of revascularization of the ulcerated foot in patients with diabetes and peripheral artery disease: A systematic review. Diabetes Metab Res Rev, 2016; 32(Suppl. 1): 136–44
3. Yi J, Gao ZF: MicroRNA-9-5p promotes angiogenesis but inhibits apoptosis and inflammation of high glucose-induced injury in human umbilical vascular endothelial cells by targeting CXCR4. Int J Biol Macromol, 2019; 130: 1–9
4. Wong TY, Cheung CM, Larsen M et al: Diabetic retinopathy. Nat Rev Dis Primers, 2016; 2: 16012
5. Al-Kharashi AS: Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy. Saudi J Ophthalmol, 2018; 32(4): 318–23
6. Dehdashtian E, Mehrzadi S, Yousefi B et al: Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress. Life Sci, 2018; 193: 20–33
7. Capitao M, Soares R: Angiogenesis and inflammation crosstalk in diabetic retinopathy. J Cell Biochem, 2016; 117(11): 2443–53
8. Behl T, Kaur I, Goel H, Kotwani A: Significance of the antiangiogenic mechanisms of thalidomide in the therapy of diabetic retinopathy. Vascul Pharmacol, 2017; 92: 6–15
9. Xu C, Zhao H, Chen H, Yao Q: CXCR4 in breast cancer: Oncogenic role and therapeutic targeting. Drug Des Devel Ther, 2015; 9: 4953–64
10. Dewan MZ, Ahmed S, Iwasaki Y et al: Stromal cell-derived factor-1 and CXCR4 receptor interaction in tumor growth and metastasis of breast cancer. Biomed Pharmacother, 2006; 60(6): 273–76
11. Busillo JM, Benovic JL: Regulation of CXCR4 signaling. Biochim Biophys Acta, 2007; 1768(4): 952–63
12. Unoki N, Murakami T, Nishijima K et al: SDF-1/CXCR4 contributes to the activation of tip cells and microglia in retinal angiogenesis. Invest Ophthalmol Vis Sci, 2010; 51(7): 3362–71
13. Petit I, Jin D, Rafii S: The SDF-1-CXCR4 signaling pathway: A molecular hub modulating neo-angiogenesis. Trends Immunol, 2007; 28(7): 299–307
14. Ling X, Spaeth E, Chen Y et al: The CXCR4 antagonist AMD3465 regulates oncogenic signaling and invasiveness *in vitro* and prevents breast cancer growth and metastasis *in vivo*. PLoS One, 2013; 8(3): e58426
15. Hinton CV, Avraham S, Avraham HK: Contributions of integrin-linked kinase to breast cancer metastasis and tumourigenesis. J Cell Mol Med, 2008; 12(5a): 1517–26
16. Ali MM, Kumar S, Shankar A et al: Effects of tyrosine kinase inhibitors and CXCR4 antagonist on tumor growth and angiogenesis in rat glioma model: MRI and protein analysis study. Transl Oncol, 2013; 6(6): 660–69
17. Muralidharan R, Panneerselvam J, Chen A et al: HuR-targeted nanotherapy in combination with AMD3100 suppresses CXCR4 expression, cell growth, migration and invasion in lung cancer. Cancer Gene Ther, 2015; 22(12): 581–90
18. Trudeau K, Molina AJ, Guo W, Roy S: High glucose disrupts mitochondrial morphology in retinal endothelial cells: Implications for diabetic retinopathy. Am J Pathol, 2010; 177(1): 447–55
19. Sheu ML, Ho FM, Yang RS et al: High glucose induces human endothelial cell apoptosis through a phosphoinositide 3-kinase-regulated cyclooxygenase-2 pathway. Arterioscler Thromb Vasc Biol, 2005; 25(3): 539–45
20. Wang M, Hu J, Yan L et al: High glucose-induced ubiquitination of G6PD leads to the injury of podocytes. FASEB J, 2019; 33(5): 6296–310
21. Ho FM, Lin WW, Chen BC et al: High glucose-induced apoptosis in human vascular endothelial cells is mediated through NF-kappaB and c-Jun NH2-terminal kinase pathway and prevented by PI3K/Akt/eNOS pathway. Cell Signal, 2006; 18(3): 391–99
22. Madonna R, Giovannelli G, Confalone P et al: High glucose-induced hyperosmolarity contributes to COX-2 expression and angiogenesis: Implications for diabetic retinopathy. Cardiovasc Diabetol, 2016; 15: 18
23. Madonna R, Geng YJ, Shelat H et al: High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1. Biochim Biophys Acta, 2014; 1842(11): 2266–75
24. Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C: Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr Diabetes Rev, 2006; 2(1): 71–98
25. Patel JI, Tombran-Tink J, Hykin PG et al: Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: Implications for structural differences in macular profiles. Exp Eye Res, 2006; 82(5): 798–806
26. Fernando KHN, Yang HW, Jiang Y et al: Diphlorethohydroxycarmalol isolated from *Ishige okamurae* represses high glucose-induced angiogenesis *in vitro* and *in vivo*. Mar Drugs, 2018; 16(10): pii: E375
27. Shao J, Yao Y: Transthyretin represses neovascularization in diabetic retinopathy. Mol Vis, 2016; 22: 1188–97
28. Wen J, Zhang JQ, Huang W, Wang Y: SDF-1alpha and CXCR4 as therapeutic targets in cardiovascular disease. Am J Cardiovasc Dis, 2012; 2(1): 20–28
29. Wang L, Guo S, Zhang N et al: The role of SDF-1/CXCR4 in the vasculogenesis and remodeling of cerebral arteriovenous malformation. Ther Clin Risk Manag, 2015; 11: 1337–44
30. Yin Y, Duan J, Guo C et al: Danshensu accelerates angiogenesis after myocardial infarction in rats and promotes the functions of endothelial progenitor cells through SDF-1alpha/CXCR4 axis. Eur J Pharmacol, 2017; 814: 274–82
31. Yu X, Chen D, Zhang Y et al: Overexpression of CXCR4 in mesenchymal stem cells promotes migration, neuroprotection and angiogenesis in a rat model of stroke. J Neurol Sci, 2012; 316(1–2): 141–49
32. Pozzobon T, Goldoni G, Viola A, Molon B: CXCR4 signaling in health and disease. Immunol Lett, 2016; 177: 6–15
33. Fricker SP, Anastassov V, Cox J et al: Characterization of the molecular pharmacology of AMD3100: A specific antagonist of the G-protein coupled chemokine receptor, CXCR4. Biochem Pharmacol, 2006; 72(5): 588–96
34. Hu JS, Freeman CM, Stolberg VR et al: AMD3465, a novel CXCR4 receptor antagonist, abrogates schistosomal antigen-elicited (type-2) pulmonary granuloma formation. Am J Pathol, 2006; 169(2): 424–32
35. Zhang T, Yang F, Li W et al: Suppression of the SDF1/CXCR4/betacatenin axis contributes to bladder cancer cell growth inhibition *in vitro* and *in vivo*. Oncol Rep, 2018; 40(3): 1666–74
36. Shen CC, Chen B, Gu JT et al: AMD3100 treatment attenuates pulmonary angiogenesis by reducing the c-kit (+) cells and its pro-angiogenic activity in CBDL rat lungs. Biochim Biophys Acta Mol Basis Dis, 2018; 1864(3): 676–84
37. Mrugacz M, Bryl A, Bossowski A: Neuroretinal apoptosis as a vascular dysfunction in diabetic patients. Curr Neuropharmacol, 2016; 14(8): 826–30
38. Yin Y, Chen F, Wang W et al: Resolvin D1 inhibits inflammatory response in STZ-induced diabetic retinopathy rats: Possible involvement of NLRP3 inflammasome and NF-kappaB signaling pathway. Mol Vis, 2017; 23: 242–50
39. Soufi FG, Mohammad-Nejad D, Ahmadieh H: Resveratrol improves diabetic retinopathy possibly through oxidative stress – nuclear factor κB – apoptosis pathway. Pharmacol Rep, 2012; 64(6): 1505–14

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