

Puerarin regulates neovascular glaucoma through pigment epithelium-derived growth factor-induced NF- κ B signaling pathway

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Abstract. Neovascular glaucoma is an ophthalmic disease and a potentially blinding secondary glaucoma caused by the formation of abnormal new blood vessels on the iris, which can prevent the normal drainage of water from the anterior segment of the eye. Evidence from China has suggested that puerarin benefits many diseases including myocardial infarction, stable angina, cerebral ischemia and glaucoma in a clinical setting. In the present study, the aim was to investigate the efficacies of puerarin on neovascular glaucoma in a mouse model. The molecular mechanism of puerarin-mediated treatment for neovascular glaucoma was also investigated both *in vitro* and *in vivo*. Inflammatory responses in mice with neovascular glaucoma were analyzed by western blotting. Oxidative stress levels were investigated following treatment with puerarin in a mouse model of neovascular glaucoma. The results indicated that puerarin markedly improved growth of vascular endothelial cells. The present study reported that puerarin treatment markedly decreased interleukin (IL)-1 β , IL-17A and tumor necrosis factor- α expression levels in mice with neovascular glaucoma. It was found that puerarin significantly decreased oxidative stress levels by reducing reactive oxygen species, superoxide dismutase and malondialdehyde levels, as well as neuronal nitric oxide synthase (NOS) and inducible NOS expression levels. Results indicated that expression levels of pigment epithelium-derived growth factor were significantly inhibited following treatment with puerarin. Mechanism analysis demonstrated that treatment with puerarin effectively inhibited nuclear factor (NF)- κ B activity and its target

protein levels p65, inhibitor of NF- κ B kinase subunit β and inhibitor of NF- κ B kinase subunit α in vascular endothelial cells. Increasing endothelial-derived growth factor (EDGF) expression levels could stimulate NF- κ B activity and abolish the inhibitory effects of puerarin. An animal study reported that puerarin treatment presented therapeutic effects for mice with neovascular glaucoma. Numbers of new vessels in iris were recovered to normal following puerarin treatment. In conclusion, these results indicated that puerarin treatment can inhibit inflammatory responses and oxidative stress, platelet-derived growth factor (PDGF) expression and NF- κ B activity, suggesting puerarin may be a potential agent for the treatment of neovascular glaucoma through PDGF-induced NF- κ B signaling pathway.

Introduction

Neovascular glaucoma is a serious ophthalmic disease that affects visual sense and leads to open-angle glaucoma and angle closure glaucoma (1). Neovascular glaucoma presents a potentially blinding secondary glaucoma caused by the formation of abnormal new blood vessels on iris, which can prevent normal drainage of aqueous liquid from the anterior segment of the eye (2,3). There are >40 different diseases that can lead to the cause of neovascular glaucoma, which are almost extensive involvement in posterior section of oxygen or localized front section of oxygen (4). Studies have suggested that central retinal vein occlusion, diabetic retinopathy and other diseases are the most common inducer of neovascular glaucoma (5,6). Mechanisms of the initiation and progression of neovascular glaucoma have been widely investigated and evidence indicates that imbalance of new vessels in iris area contributes to the initiation of neovascular glaucoma (7). Therefore, many theories have been proposed for explaining the neovascularization in the progression of neovascular glaucoma.

Currently, various therapeutic schedules for neovascular glaucoma have been put forward including physical and drug therapies (8). Physical treatment includes panretinal photocoagulation, retina frozen and iris-cornea-angle photocoagulation (9). Drug treatments include hormonal drugs and antibody drugs (10,11). In previous years, a

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large numbers of traditional Chinese medicine have been investigated for the treatment of ophthalmic diseases and presented many benefits and encouraging comprehensive effects on hypertension in eyes (12). This study has stimulated scientists to investigate the mechanisms of traditional Chinese medicine-mediated treatments for human diseases. Treatments of neovascular glaucoma by using traditional Chinese medicine have been widely accepted and achieved clinical outcomes (13). In the current study, the authors investigated the efficacy of puerarin for the treatment of neovascular glaucoma in a mice model.

Puerarin, a natural flavonoid, is a major isoflavonoid compound extracted from *Pueraria* and exhibits potential therapeutic effects for many human diseases (14,15). Puerarin has been reported to have many benefits and medicinal properties (16). In addition, puerarin can attenuate amyloid- β -induced cognitive impairment through suppression of apoptosis in the rat hippocampus *in vivo* (17). A previous study reported that puerarin can prevent the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead (18).

Furthermore, the synthesis of puerarin derivatives and their protective effect on the myocardial ischemia and reperfusion injury also have been examined in animal experiments (19). The present study design investigated the therapeutic effects of puerarin for the treatment of neovascular glaucoma in a mouse model and results indicated that puerarin significantly improved symptoms of neovascular glaucoma in a 30-day treatment period.

In the present study, the purpose was to research the efficacy of puerarin for neovascular glaucoma and potential mechanisms of puerarin-mediated signaling pathway in the process of neovascularization in the progression of neovascular glaucoma. In addition, the authors analyzed inflammatory responses and oxidative stress in mice with neovascular glaucoma. Notably, the authors also studied the NF- κ B signaling pathway in vascular endothelial cells located on iris area in mice with neovascular glaucoma. The authors aimed to explain the mechanism by which puerarin contributes to prevention of neovascularization induced by hypoxia through inhibition of the pigment epithelium-derived growth factor-induced NF- κ B signaling pathway.

Materials and methods

Ethical statement. The current study was approved by the ethics committee of Tianjin Medical University College of Optometry (Tianjin, China). All surgery and euthanasia were made to minimize suffering.

Cells culture and reagents. Vascular endothelial cells on iris area were isolated from experimental mice and cultured in minimum essential medium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) supplemented with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Vascular endothelial cells were cultured in a 37°C humidified atmosphere of 5% CO₂.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total mRNA was obtained from vascular

endothelial cells on iris area by using RNAeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). Expression levels of nNOS and iNOS in vascular endothelial cells were determined by qRT-PCR using TaqPath™ 1-Step RT-qPCR Master Mix, CG (Applied Biosystems™) according to manufacturer's instructions (20). All the forward and reverse primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. (nNOS forward, 5'-CGGCTGTGCTTTAATGGAGAT-3' and reverse, 5'-GAGGAGACGCTGTTGAATCG-3'; iNOS forward, 5'-AAGGATGGGAAGTACTGG-3' and reverse, 5'-CAGTGTTCCTCAGAAAGAG-3'; ROS forward, 5'-GAGCCTTCTGGTGAAAATCAA-3' and reverse, 5'-ACG ATTCAGCTCATCCTCCA-3'; β -actin forward, 5'-AGAGCT ACGAGCTGCCTGA-3' and reverse, 5'-AGCACTGTGTTG GCGTACAG-3'). The PCR conditions were 95°C for 15 min, and 95°C for 10 sec, followed by 40 cycles by 54°C for 20 sec and 72°C for 20 sec. Relative mRNA expression level changes were calculated by 2^{- $\Delta\Delta$ C_q} (21). The results are expressed as the n-fold way compared to control.

Western blotting. Vascular endothelial cells were on iris area isolated from experimental mice and homogenized in lysate buffer containing protease-inhibitor (Sigma-Aldrich; Merck KGaA) and were centrifuged at 8,000 x g at 4°C for 10 min. The supernatant of the mixture were used for analysis of the protein. 12.5% SDS-PAGE assays were performed as previously described (22). Proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (Merck Millipore). For western blotting, primary goat anti-mouse antibodies: p65 (1:1,000, ab32536), IKK- β (1:1,000, ab124957), I κ B α (1:1,000, ab109300), vascular endothelial cell growth factor (VEGF; 1:1,000, ab32152), TGF- β (1:1,000, ab31013), angiogenin (1:1,000, ab139947), Neurotrophin 3 (1:1,000, ab53685) VSF (1:1,000, ab9695), IL-1 β (1:1,000, ab200478), IL-17 (1:1,000, ab79056), IFN- γ (1:1,000, ab32152), SOD (1:1,000, ab137037), malondialdehyde (1:1,000, ab6463) and β -actin (1:1,000, ab8227) (all from Abcam, Cambridge, UK) were added following blocking (5% skimmed milk) for 60 min at 37°C. Following washing with PBS three times, the secondary rabbit anti-goat antibodies goat anti-rabbit IgG mAb (1:5,000, PV-6001, ZSGB-BIO; OriGene Technologies, Beijing, China) were used to detect purpose protein for 2 h at 37°C. The results were visualized by using enhanced chemiluminescence substrate ECL Select™ Ventana Benchmark automated staining system (Roche Diagnostics, Basel, Switzerland).

Animal study. A total of 20 female C57BL/6 mice (8 weeks, 25-30 g body weight) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and housed with a 12 h light-dark artificial cycle. All mice were free to access food and water. To detect the efficacy of puerarin on neovascular glaucoma, mice model of neovascular glaucoma was established by mutation in collagen 8A2 according to a previous study (23). Mice with neovascular glaucoma were divided into two groups (n=10/group) and received treatment with puerarin (0.2 mg/kg) with PBS as negative control.

Immunohistochemical staining. Immunohistochemical staining was performed by an avidin-biotin-peroxidase technique. Paraffin-embedded tumor tissue sections were prepared

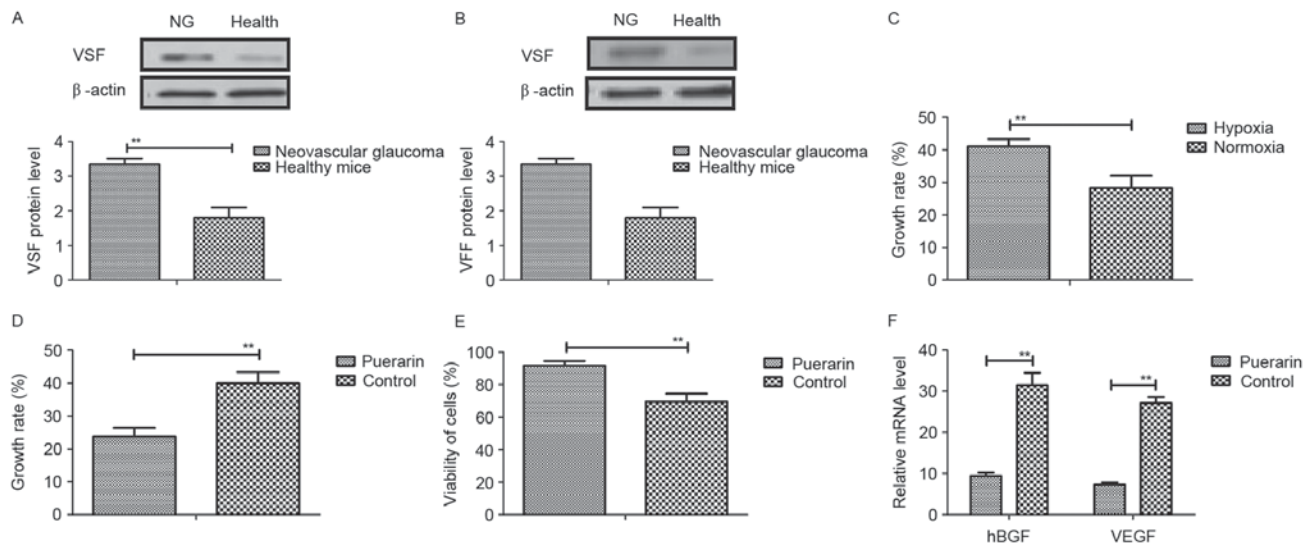


Figure 1. Effects of puerarin on growth and viability of vascular endothelial cells induced by VSF in hypoxia. (A and B) Expression levels of (A) VSF and (B) VFF in vascular endothelial cells in mice with neovascular glaucoma. (C) Growth of vascular endothelial cells induced by VSF. (D) Growth of vascular endothelial cells following treatment with puerarin in hypoxia. (E) Viability of vascular endothelial cells treated by puerarin in normoxia. (F) Expression levels of hBGF and VEGF in vascular endothelial cells. One-way analysis of variance or two-tailed Student's t-test revealed a significant effect. Data are expressed as the mean \pm standard error of the mean. ** $P < 0.01$ vs. control. VSF, vaso-stimulating factor; VFF, vaso-formative factor; hBGF, heparin-binding growth factors; VEGF, vascular endothelial growth factor.

and epitope retrieval was performed for further analysis. The paraffin sections were subjected with hydrogen peroxide (3%) for 10-15 min, which subsequently were blocked by a regular blocking solution (5% skimmed milk) for 10-15 min at 37°C. Finally, the sections were incubated in anti-p65 (1:1,000, ab32536), anti-IKK- β (1:1,000, ab124957), anti-I κ B α (1:1,000, ab109300), anti-VEGF (1:1,000, ab32152), anti-TGF- β (1:1,000, ab31013) and anti-angiogenin (1:1,000, ab139947) (all from Abcam), respectively, at 4°C for 12 h following blocking. All sections were washed three times and incubated with secondary antibodies for 1 h at 37°C and were observed by six random fields of view under the microscope.

Activity of NF- κ B. Activity of NF- κ B in vascular endothelial cells from healthy mice and mice with neovascular glaucoma were analyzed following treatment with puerarin. The activity of NF- κ B was conducted according to previous study (24).

Inflammatory response analysis. Inflammatory responses in serum of mice with neovascular glaucoma were analyzed after treatment with puerarin. Percentage of numbers of lymphocytes, monocytes and neutrophils were examined by flow cytometry according to a previous study (25).

Statistical analysis. All data are presented as mean \pm standard error of the mean, are conducted in triplicate and performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences between experimental groups were analyzed by Student's t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Puerarin inhibits aberrant growth of vascular endothelial cells induced by vaso-stimulating factor in hypoxia. The

authors first detected the effects of puerarin on vascular endothelial cells *in vitro*. The expression levels of vaso-stimulating factor (VSF) and vaso-formative factor (VFF) were upregulated in mice with neovascular glaucoma compared to healthy mice (Fig. 1A and B). In addition, as presented in Fig. 1C, VSF could induce vascular endothelial cells growth under hypoxic conditions, compared to normoxia. However, the authors found that puerarin significantly inhibited growth of vascular endothelial cells induced by VSF in hypoxia (Fig. 1D). Furthermore, it was observed that puerarin improved viability of vascular endothelial cells in normoxia (Fig. 1E). Moreover, results suggested that heparin-binding growth factors and VEGF mRNA expression levels were inhibited in vascular endothelial cells induced VSF (Fig. 1F). Taken together, these results suggested that puerarin can inhibit aberrant growth of vascular endothelial cells induced by VSF in hypoxia, which may be beneficial for neovascular glaucoma.

Puerarin suppresses expression levels inflammatory responses and factors in mice with neovascular glaucoma. The authors next analyzed the anti-inflammatory effects of puerarin in mice with neovascular glaucoma *in vivo*. Interleukin (IL)-1 β , IL-17A and tumor necrosis factor (TNF)- α expression levels were increased in vascular endothelial cells induced by neovascular glaucoma, whereas puerarin treatment may significantly decreased upregulation of IL-1 β , IL-17A and TNF- α *in vitro* (Fig. 2A-C). In addition, the authors observed that numbers of lymphocytes, monocytes and neutrophils were upregulated and puerarin could become downregulated in serum of mice with neovascular glaucoma (Fig. 2D-F). Furthermore, it was observed that interferon- γ and neurotrophic factor expression levels were increased in vascular endothelial cells in mice with neovascular glaucoma following treatment with

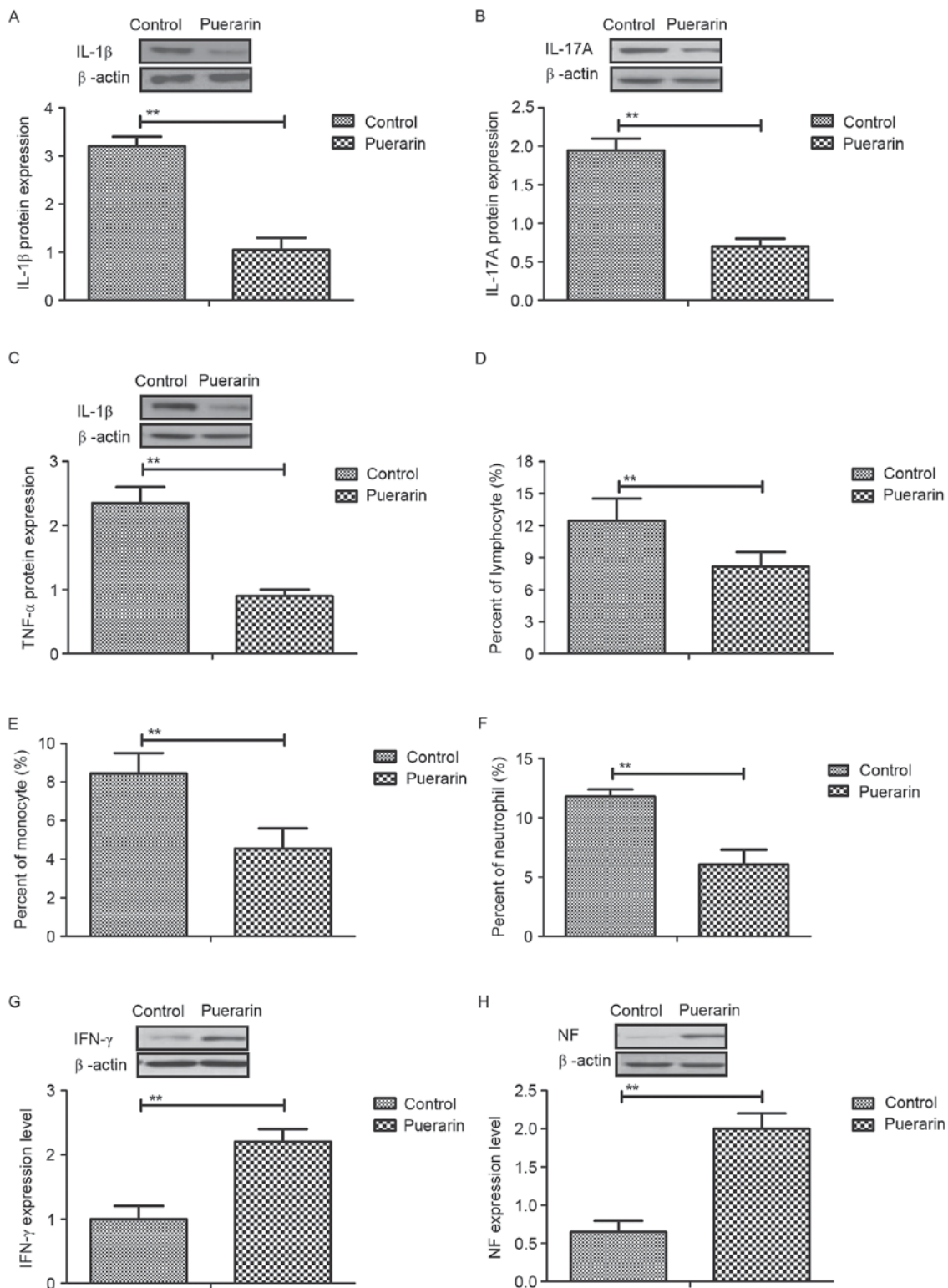


Figure 2. Analysis of inflammatory responses and factors in mice with neovascular glaucoma after puerarin treatment. (A-C) Protein expression levels of (A) IL-1 β , (B) IL-17A and (C) TNF- α in vascular endothelial cells induced by neovascular glaucoma *in vivo*. (D-F) Percentage of (D) lymphocytes, (E) monocytes and (F) neutrophils was analyzed of in serum in mice with neovascular glaucoma following treatment with puerarin. (G and H) IFN- γ and NF expression levels in vascular endothelial cells in mice with neovascular glaucoma following treatment with puerarin. One-way analysis of variance or two-tailed Student's t-test revealed a significant effect. Data are expressed as the mean \pm standard error of the mean. ** $P < 0.01$ vs. control. IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ ; NF, neurotrophic factor neurotrophin 3.

puerarin (Fig. 2G and H). Collectively, the results suggested that puerarin can suppress expression levels inflammatory responses and factors in mice with neovascular glaucoma both *in vitro* and *in vivo*.

Puerarin attenuates oxidative stress in vascular endothelial cells and in mice with neovascular glaucoma. A previous study suggested that neovascular glaucoma is associated with oxidative stress (26). Therefore, the authors analyzed the effects of

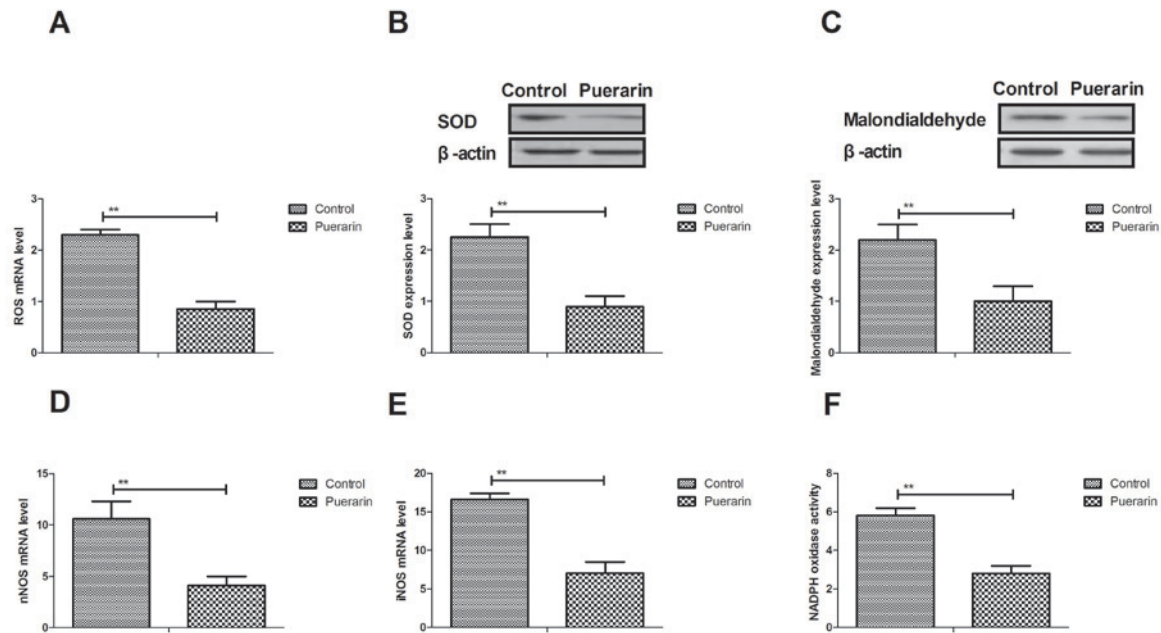


Figure 3. Effects of puerarin on oxidative stress in vascular endothelial cells and in mice with neovascular glaucoma. (A-C) Protein expression levels of (A) ROS, (B) SOD and (C) malondialdehyde levels in vascular endothelial cells. (D and E) Analysis of mRNA expression levels of (D) nNOS and (E) iNOS levels in vascular endothelial cells. (F) NADPH oxidase activity in vascular endothelial cells in mice with neovascular glaucoma following puerarin treatment. One-way analysis of variance or two-tailed Student's t-test revealed a significant effect. Data are expressed as the mean \pm standard error of the mean. **P<0.01 vs. control. ROS, reactive oxygen species; SOD, superoxide dismutase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase.

puerarin on changes of oxidative stress in vascular endothelial cells and in mice with neovascular glaucoma. As illustrated in Fig. 3A-C, puerarin significantly decreased oxidative stress levels by reducing reactive oxygen species (ROS), superoxide dismutase (SOD) and malondialdehyde levels in vascular endothelial cells *in vitro*. In addition, neuronal nitric oxide synthase (NOS) and inducible NOS mRNA expression levels were markedly decreased by puerarin in vascular endothelial cells (Fig. 3D and E). Furthermore, NADPH oxidase activity was improved by puerarin in vascular endothelial cells in mice with neovascular glaucoma compared to the control (Fig. 3F). Collectively, the results suggested that puerarin can attenuate oxidative stress in vascular endothelial cells and in mice with neovascular glaucoma.

Puerarin regulates aberrant growth of vascular endothelial cells through platelet derived growth factor (PDGF)-induced NF- κ B signaling pathway. In order to identify the mechanism of puerarin-mediated neovascular glaucoma, the authors analyzed the NF- κ B signaling pathway and the target proteins in vascular endothelial cells. As presented in Fig. 4A-C, puerarin markedly inhibited expression levels of p65, inhibitor of NF- κ B kinase subunit β (IKK- β) and inhibitor of NF- κ B kinase subunit α (IkB α) in vascular endothelial cells. In addition, NF- κ B activity was inhibited by puerarin treatment in vascular endothelial cells (Fig. 4D). Puerarin decreased PDGF expression, and restoration of PDGF expression abolished puerarin-inhibited NF- κ B activity (Fig. 4E). In addition, PDGF restoration abolished the beneficial effects of puerarin on inflammatory responses and oxidative stress (Fig. 4F and G). Furthermore, PDGF restoration also promoted

TGF- β and VEGF expression levels (Fig. 4H). These results suggested that puerarin can regulate aberrant growth of vascular endothelial cells through the PDGF-induced NF- κ B signaling pathway.

Puerarin shows inhibition of aberrant neovascularization in mice with neovascular glaucoma. To analyze the therapeutic effects of puerarin for neovascular glaucoma, a mouse model of neovascular glaucoma was established by mutation in collagen 8A2 (23). Intraocular pressure of experimental mice was measured for examine the therapeutic efficacy of puerarin. As demonstrated in Fig. 5A, intraocular pressure was significantly improved by puerarin treatment. It was also observed aberrant vascular proliferation was inhibited in mice with neovascular glaucoma treated by puerarin (Fig. 5B). In addition, fluorescence staining showed that p65, IKK- β and IkB α expression levels were significantly downregulated by puerarin treatment in vascular endothelial cells (Fig. 5C). Furthermore, the results indicated that expression levels of VEGF, TGF- β and angiogenin were downregulated in vascular endothelial cells in puerarin-treat mice (Fig. 5D). Histological analysis indicated that neovascularization and retinal degeneration were improved in mice with neovascular glaucoma following treatment with puerarin (Fig. 5E and F). Importantly, visual function and retinal whole-mount analysis suggested that puerarin could markedly improve visual function and pathological hemorrhages in mice with neovascular glaucoma (Fig. 5G and H). Taken together, these results puerarin presented benefits for the inhibition of aberrant neovascularization and improves pathological symptoms in mice with neovascular glaucoma.

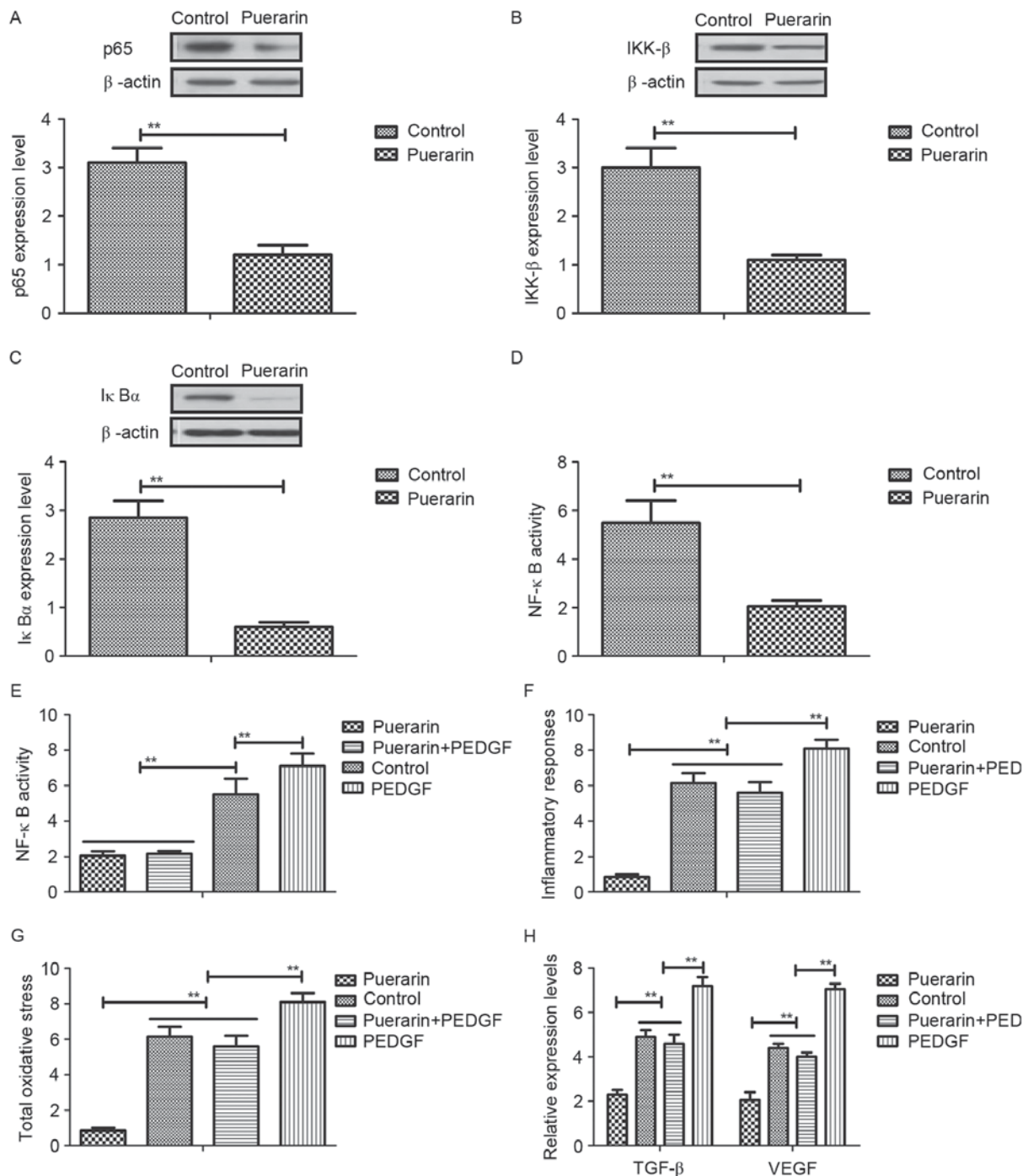


Figure 4. Puerarin mediated aberrant growth of vascular endothelial cells through PDGF-induced NF- κ B signal pathway. (A-C) Expression levels of (A) p65, (B) IKK- β and (C) I κ B α in vascular endothelial cells following puerarin treatment. (D) NF- κ B activity in vascular endothelial cells following puerarin treatment. (E) Analysis of PDGF expression in vascular endothelial cells following treatment with puerarin. (F) Inflammatory responses and (G) oxidative stress in vascular endothelial cells were analyzed by PDGF restoration. (H) TGF- β and VEGF expression levels in vascular endothelial cells following PDGF restoration. One-way analysis of variance or two-tailed Student's t-test revealed a significant effect. ** $P < 0.01$ vs. control. Data are expressed as the mean \pm standard error of the mean. IKK β , inhibitor of NF- κ B kinase subunit β ; I κ B α , inhibitor of NF- κ B kinase subunit α ; NF- κ B, nuclear factor- κ B; TGF- β , tumor necrosis factor- β ; VEGF, vascular endothelial growth factor. PDGF, platelet derived growth factor.

Discussion

Neovascular glaucoma is a disease that new fiber vascular membrane is overgrowth of the iris and trabecular, which leads to fibrous thickening of the filtration membrane in the operation area and the peripheral anterior synechia of the iris area (27). Neovascular glaucoma is also known as hemorrhagic

glaucoma due to new blood vessels rupture and recurrent anterior chamber bleeding, which has been considered as intractable ophthalmic diseases (28). Therefore, treatments for neovascular glaucoma has become a nightmare for patients in clinical. Although many treatments have proposed for patients with neovascular glaucoma, the efficacy is limited for patients and the recurrence rate is higher following patients

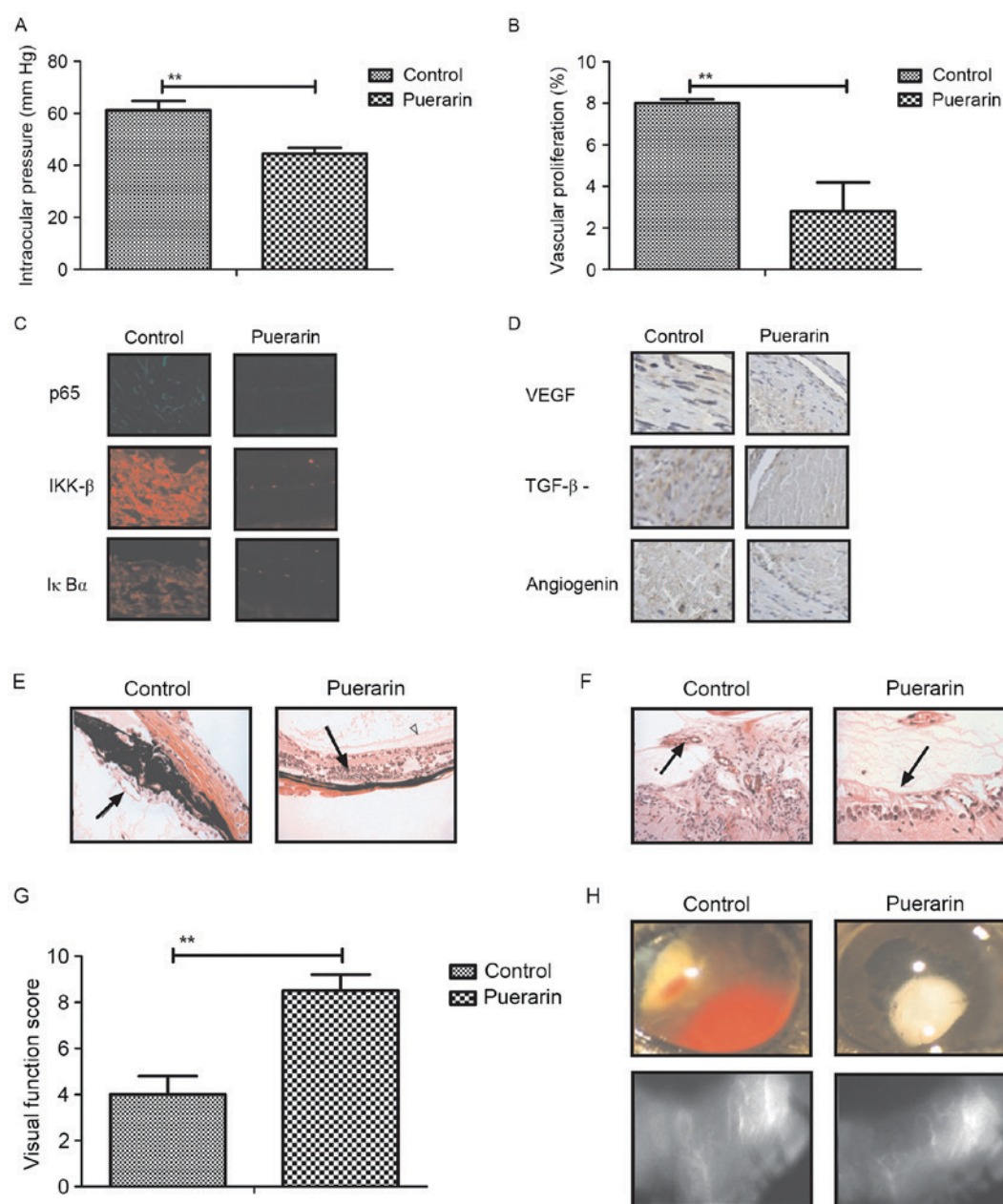


Figure 5. *In vivo* effects of puerarin on aberrant neovascularization in mice with neovascular glaucoma. (A) Evaluation of intraocular pressure in mice with neovascular glaucoma. (B) Vascular proliferation in mice with neovascular glaucoma following treatment with puerarin. (C) Expression levels of p65, IKK- β and I κ B α in vascular endothelial cells determined by immunostaining. Magnification, x20. (D) Expression levels of VEGF, TGF- β and angiogenin in vascular endothelial cells determined by histological staining. Magnification, x20. (E) Neovascularization and (F) retinal degeneration was analyzed in iris area in mice with neovascular glaucoma. Magnification, x40. (G and H) Improvement of visual function and pathological hemorrhages were examined in mice with neovascular glaucoma. Magnification, x5. One-way analysis of variance or two-tailed Student's t-test revealed a significant effect. Data are expressed as the mean \pm standard error of the mean. ** $P < 0.01$ vs. control. IKK β , inhibitor of NF- κ B kinase subunit β ; I κ B α , inhibitor of NF- κ B kinase subunit α ; VEGF, vascular endothelial growth factor; TGF- β , tumor necrosis factor- β .

receiving clinical treatments (29). In the present study, the authors introduced an efficient drug of puerarin for the treatment of neovascular glaucoma in an animal model. Not only have the therapeutic effects of neovascular glaucoma been investigated, but also elaborated the molecular mechanism of puerarin-mediated improvement of neovascular glaucoma. Our results suggested that puerarin can inhibit aberrant growth of vascular endothelial cells, suppress inflammatory responses and factors and oxidative stress in vascular endothelial cells and in mice with neovascular glaucoma. Importantly, the findings indicated that puerarin regulates growth of vascular

endothelial cells, neovascularization and retinal degeneration through PDGF-induced NF- κ B signaling pathway in mice with neovascular glaucoma.

Aberrant growth of vascular endothelial cells contributes to the deterioration of vascular proliferation and visual function for patients with neovascular glaucoma (30). As is known, inhibition of aberrant growth of vascular endothelial cells is a potential treatment for patients with neovascular glaucoma (9). In previous years, anti-vascular endothelial growth factor for neovascular glaucoma therapy has been prevalent and achieves efficacy for patients with neovascular glaucoma (31). However,

long-term treatment of anti-vascular endothelial growth factor obviously reduces pharmacodynamics of the antibody (32). The outcomes suggested that puerarin can inhibit the aberrant growth of vascular endothelial cells and avoid the defects of anti-vascular endothelial growth factor antibody, which presents an ideal agent in anti-glaucoma therapy.

Inflammatory responses have been identified associated with the initiation and progression of glaucoma (33). Chang *et al* (34) suggested increasing mast cell numbers as a possible indicator of preoperative glaucoma surgery inflammation was observed in the conjunctiva of glaucoma patients. In addition, Furtado *et al* (35) have showed that inflammation in patients under topical glaucoma is an indicator following treatment with indication to surgery. Vohra *et al* (36) have indicated that the role of inflammation in the pathogenesis of glaucoma. Furthermore, evidences have indicated that other factors such as genetics, excitotoxicity and blood flow can act as potential causal factors for progressive of glaucoma (37). The current results demonstrated that puerarin could inhibit inflammatory responses in mice with neovascular glaucoma. These inhibitory effects of IL-1 β , IL-17A, TNF- α , lymphocytes, monocytes and neutrophils on experimental mice are beneficial for the recovery of neovascular glaucoma. Interestingly, the authors also observed that oxidative stress is downregulated by puerarin treatment.

Previous studies have reported that oxidative-antioxidative balance is associated with progression of neovascular glaucoma and that the resulting oxidative stress constitutes a major contribution involved in the pathophysiology of neovascular glaucoma (38). Research has indicated that retinal pigment epithelial damage induces more production of PDGF and breaks the blood-retinal barrier, and retinal inflammation in dogs with primary glaucoma (39). Oxidative stress can regulate the activity of complement in human glaucoma (40). These results simultaneously suggested that decreasing of oxidative stress in vascular endothelial cells may be contributed to the inhibition of aberrant growth of vascular endothelial cells. The results revealed that puerarin can markedly suppress ROS, SOD and malondialdehyde levels in vascular endothelial cells in experimental mice. Downregulation of oxidative stress contributes to the decreasing of intraocular pressure. Notably, this downregulation is regulated by the NF- κ B signaling pathway in mice with neovascular glaucoma.

The NF- κ B signaling pathway involves in various processes of neovascular glaucoma. In the present study, the authors investigated the relationships between the NF- κ B pathway and neovascular glaucoma in a mouse model following treatment with puerarin. A previous study has suggested that the NF- κ B pathway can regulate the activation of NLRP3 and caspase-8 inflammasomes in acute glaucoma (41). In addition, NF- κ B-mediated inflammatory stress response may be predictor of the glaucoma marker SELE in trabecular meshwork cells (42). In the present study, puerarin treatment significantly inhibits NF- κ B expression and activity in vascular endothelial cells in experimental mice. Stimulation of NF- κ B activity canceled the inhibitory effects of puerarin for inflammatory responses and oxidative stress.

In conclusion, the study investigated the efficacy and potential mechanism of puerarin-mediated improvement of

neovascular glaucoma in a mice model. Puerarin treatment not only inhibits aberrant growth of vascular endothelial cells, inflammatory responses and oxidative stress, but also improves neovascularization, retinal degeneration and visual function in mice with neovascular glaucoma. Most importantly, the NF- κ B pathway is involved in puerarin-inhibited inflammatory responses and oxidative stress is observed *in vitro* and *in vivo*. These outcomes suggested that puerarin is beneficial for the treatment of neovascular glaucoma through regulation of the PDGF-induced NF- κ B signaling pathway.

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