

UDC 573.6

OBTAINING OF AN EXPERIMENTAL RETINOPATHY MODEL IN RABBITS USING REPEATED INTRAVITREAL ADMINISTRATION OF VEGF AND bFGF

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ABSTRACT

Retinopathy is one of the causes of early vision loss among the working population. Retinopathy is a common complication of diabetes. However, preclinical testing of new therapies in animal models of diabetic retinopathy remains complicated by the long time to symptom onset and the high cost of animal housing. One of the solutions is to create models similar to retinopathy without inducing diabetes. One of the approaches for such method is to induce retinopathy using factors such as vascular endothelial growth factor and basic fibroblast growth factor. In our study, we tested the ability of a combination of these factors at low concentrations to induce a stable retinopathy model. The results showed that, despite the fact that the concentration of the factors was reduced, repeated administration of them resulted in a model exhibiting all the key features of the disease.

Key words: retinopathy, rabbit model, VEGF, bFGF, optical coherence tomography.

INTRODUCTION

Pathological retinal neovascularization is a characteristic feature of several vision-threatening retinal diseases and is associated with disruption of the blood-retinal barrier, increased vascular permeability, hemorrhage, and progressive structural damage to the retina. Vascular endothelial growth factor (VEGF) is a major regulator of pathological retinal angiogenesis and vascular permeability and has therefore been widely used to establish experimental models of retinal neovascularization.

In rabbits, sustained intravitreal delivery of recombinant human VEGF has been shown to induce pronounced retinal vascular changes. Ozaki et al. demonstrated that implantation of a VEGF-containing polymeric preparation into the vitreous cavity resulted in dilation and increased tortuosity of retinal vessels within seven days, followed by vascular leakage and neovascularization within 14–21 days [1]. However, the induced neovascularization subsequently regressed as VEGF levels declined, demonstrating the dependence of the model on sustained exposure to the angiogenic stimulus.

To enhance the retinal angiogenic response, VEGF has also been combined with basic fibroblast growth factor (bFGF). Sustained intravitreal exposure to VEGF and bFGF resulted in rapid and pronounced retinal neovascularization accompanied by vascular leakage and hemorrhage in rabbits [2]. More severe retinal alterations, including tractional retinal detachment, have also been reported following sustained exposure to these two angiogenic factors [3].

Although sustained-release systems provide prolonged exposure to angiogenic factors, they require implantation of a carrier material into the vitreous cavity. Repeated direct intravitreal administration may provide an alternative approach for maintaining the angiogenic stimulus without the use of a

polymeric delivery system. Therefore, in the present study, we investigated a modified rabbit model based on three consecutive intravitreal administrations of VEGF and bFGF. The aim of the study was to establish and characterize retinal vascular changes induced by repeated administration of these two angiogenic factors without the use of a sustained-release polymer.

MATERIALS AND METHODS

Animals. All experiments were conducted using mongrel rabbits. The research protocol was approved by the Ethics Committee of the National Center for Biotechnology (No.4, 24.10.2026). The study involved mongrel rabbits aged 3 months and weighing at least 2 kg. Three animals were used in each experimental group. Only the right eye of the animals participated in the experiment; the left eye served as a control. Since, according to the manufacturer's instructions, the factors were dissolved in MQ water, nothing was introduced into the control eyes.

Induction of Retinal Vascular Changes by VEGF and bFGF. Intravitreal administration was performed under isoflurane anesthesia combined with local anesthesia using Novocain. Prior to injection, the ocular surface was disinfected with chlorhexidine solution (0,05%). VEGF (AF-100-20-10UG, Gibco, USA) and bFGF (PHG0264, Gibco, USA) were prepared according to the manufacturers' instructions. VEGF and bFGF were administered intravitreally at a dose of 400 ng in a total injection volume of 50 µL. The injection was performed 6 mm posterior to the corneal limbus. To provide repeated exposure to the angiogenic factors, intravitreal injections of VEGF and bFGF at the same dose and volume were performed on days 1, 7, and 17 of the experiment. Retinal changes were subsequently evaluated by optical coherence tomography (OCT) on days 20, 26, and 48, 60 (Figure 1).

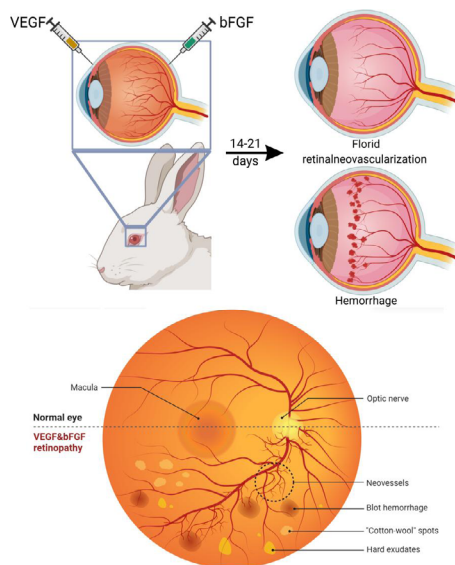


Figure 1. Experimental design of the study

Clinical Evaluation by Optical Coherence Tomography (OCT). For the analysis, animals were administered 5 mg/kg xylazine at a dose of 5 mg/kg. Examination was initiated after adequate sedation was achieved to allow safe positioning and manipulation of the animals. Imaging was performed using an Optovue solix optical coherence tomograph (Visionix, USA). OCT examinations were performed on days 20, 26, and 48, 60 after the initiation of the experiment. The first examination, on day 20, was performed immediately after the third intravitreal administration of VEGF and bFGF [4].

RESULTS

Repeated intravitreal administration of VEGF and bFGF resulted in the development of retinal abnormalities in rabbits. Following three administrations of the angiogenic factors, retinal changes consistent with the induced retinopathy model were detected by OCT and fundus examination.

On day 26 after the initiation of retinopathy induction,

fundus imaging demonstrated comparable signs and stages of retinal pathology in the experimental groups. OCT examination confirmed the persistence of retinal structural alterations at this time point (Figure 2). Based on these findings, human adipose tissue-derived pericytes were administered intravitreally on day 31 to one group of animals, while the second group received no cell treatment.

Seventeen days after pericyte administration, corresponding to day 48 of the experiment, differences in disease progression were observed between the groups. In animals receiving pericytes, OCT findings remained generally similar to those observed during the previous examination (Table 1). In contrast, animals that did not receive pericytes demonstrated further retinal alterations and complications, as detected by OCT and fundus imaging (Table 2). Although fundus examination initially indicated a similar degree of retinal involvement in both groups, OCT revealed differences in the subsequent progression of retinal structural changes.

At the later observation time point, spontaneous regression of the induced retinal changes was observed in the untreated group. By day 60 of the experiment, corresponding to 43 days after the final administration of VEGF and bFGF, almost complete retinal recovery was observed in rabbits that had not received pericytes. In contrast, retinal abnormalities remained detectable at day 60 in animals treated with pericytes (Figures 3, 4; Tables 3, 4).

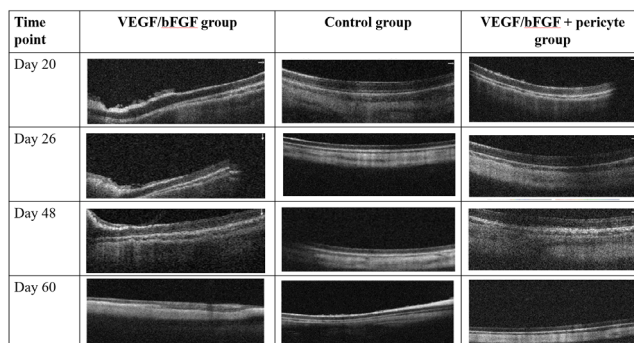


Figure 2. Representative OCT images showing retinal changes following induction of experimental retinopathy in rabbits

Table 1. Summary of OCT findings following induction of experimental retinopathy in rabbits at different time points

Time point	VEGF/bFGF group	Control group	VEGF/bFGF + pericyte group
Day 20	OD Structural alterations of the retina are visualized, retinal vessels exhibit tortuosity and variable caliber, peripapillary retinal edema is present.	OS Retinal thickness is uniform. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. No pathological changes are observed in the photoreceptor layer or retinal pigment epithelium (RPE).	OD Structural alterations of the retina are visualized, retinal vessels exhibit tortuosity and variable caliber, peripapillary retinal edema is present.
Day 26	OD Retinal thickness is uneven due to peripapillary thickening. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. The photoreceptor layer and RPE remain intact.	OS Retinal thickness is uniform. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. The photoreceptor layer and RPE remain intact.	OD Retinal thickness is uniform. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. The photoreceptor layer and RPE remain intact.

Day 48	OD Slight peripapillary retinal thickening is noted. Structural defects and degenerative changes are present within the photoreceptor layer and RPE.	OS Retinal thickness is uniform. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. The photoreceptor layer and RPE remain intact.	OD Retinal thickness is non-uniform. The neurosensory retinal architecture is well-differentiated, hyperreflective foci, inclusions, and hyporefective cavities are absent. The photoreceptor layer and RPE remain intact.
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Table 2. Summary of fundus camera findings following induction of experimental retinopathy in rabbits at different time points

Time point	VEGF/bFGF group	Control group	VEGF/bFGF + pericyte group
Day 26	OD Retinal vascular changes are observed, retinal vessels demonstrate tortuosity and caliber variation.	OS Normal retinal morphology, retinal vessels and optic nerve head appear intact.	OD Retinal vascular changes are observed, retinal vessels demonstrate tortuosity and caliber variation.
Day 48	OD Retinal vascular changes persist, retinal vessels exhibit tortuosity, caliber variation, and presence of hard and soft exudates (cotton-wool spots).	OS Normal retinal morphology, retinal vessels and optic nerve head appear intact.	OD Retinal vascular changes are observed, retinal vessels demonstrate tortuosity and caliber variation.

Table 3. Fundoscopic findings on day 60 following induction of experimental retinopathy

Fundoscopy finding	Control group	VEGF/bFGF group	VEGF/bFGF + pericyte group
Microaneurysms	absent	present	present
Microhemorrhages	absent	absent	present
Retinal Hemorrhages	absent	absent	absent
Venous dilation and tortuosity	absent	present	present
Hard exudates	absent	present	present
Soft exudates (Cotton wool spots)	absent	absent	present
IRMA	absent	present	present
Active bleeding	absent	absent	absent
Neovascularization of the disc or retina	absent	absent	absent
Hemophthalmia	absent	absent	absent

Table 4. OCT changes on day 60 following induction of experimental retinopathy

OCT changes	Control group	VEGF/bFGF group	VEGF/bFGF + pericyte group
Hyporefective dots	absent	absent	absent
Hyperreflective dots	absent	present	present
Hyporefective cavities	absent	absent	absent
Hyperreflective cavities	absent	absent	present
Neovascularization of the disc or retina	absent	absent	absent
Hemophthalmia	absent	absent	absent
Increased retinal thickness	absent	absent	absent
Soft exudates (Cotton wool spots)	absent	absent	present
Intraretinal or preretinal hemorrhage	absent	absent	absent

DISCUSSION

In the present study, repeated intravitreal administration of VEGF and bFGF induced retinal structural and vascular abnormalities in rabbits. Three administrations of 400 ng of each factor were sufficient to produce retinal changes detectable by OCT and fundus camera examination, including vascular dila-

tion and tortuosity, microaneurysms, exudative changes, and intraretinal microvascular abnormalities.

Previous rabbit models have generally used considerably higher concentrations of angiogenic factors delivered through sustained-release systems. Ozaki et al. demonstrated that sustained intravitreal release of 30 µg VEGF induced ret-

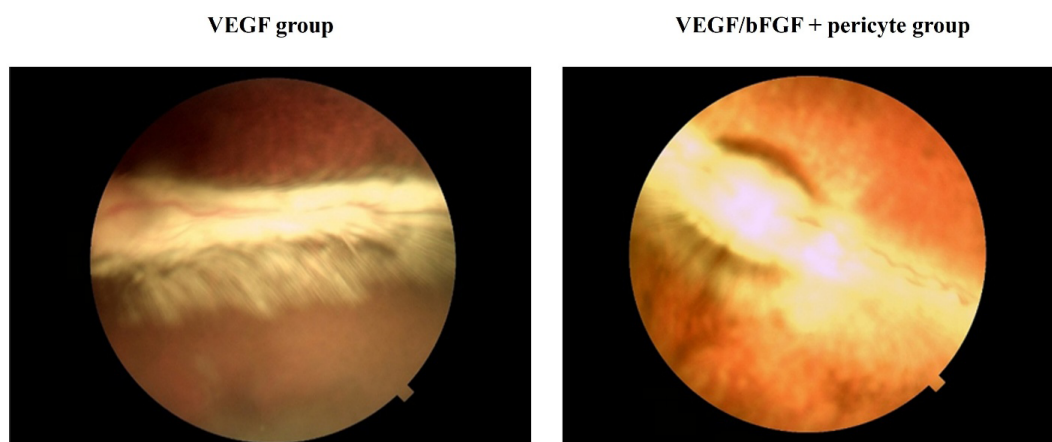


Figure 3. Representative fundus images of rabbits following induction of experimental retinopathy at day 60

inal vessel dilation and tortuosity within 7 days and retinal neovascularization within 14–21 days [1]. Wong et al. used Hydron polymer pellets containing 20 µg VEGF and 20 µg bFGF and reported rapid vascular dilation and tortuosity, followed by retinal neovascularization, hemorrhage, and, in severe cases, tractional retinal detachment [2]. Similar sustained VEGF/bFGF delivery has subsequently been used to investigate differences in retinal angiogenic responses among rabbit breeds [3]. Another study used subretinal injections of 20 ml of VEGF/Matrigel suspension (750 ng VEGF) into the optic streak, 2–3 mm below the optic disc. VEGF and Matrigel induced choroidal neovascularization and the late development of vitreoretinal inflammation, which gradually developed into proliferative vitreoretinopathy [5].

In contrast, the present model used substantially lower doses of VEGF and bFGF administered repeatedly without a polymeric sustained-release system. Despite the lower dose, retinal abnormalities persisted for several weeks. However, OCT demonstrated marked spontaneous regression of structural changes in untreated animals at the later observation period, while some vascular abnormalities remained detectable by fundus camera examination. This finding is consistent with previous evidence that maintenance of VEGF-induced retinal neovascularization depends on persistent exposure to the angiogenic stimulus [6]. Edelman et al. in their study used 200 and 500 ng of VEGF to disrupt the blood-retinal barrier by creating vascular leakage [7]. Based on these data, we decided to take 400 ng of each factor and repeated administration to induce retinopathy and showed that this model is not inferior to the polymer model, but is simpler to implement and less expensive. bFGF can regulate cell growth and differentiation and promote mitosis of retinal pigment epithelial cells, fibroblasts, and vascular endothelial cells and massive hyperplasia [8]. It may also promote the recruitment of leukocytes to sites of inflammation by enhancing the expression of adhesion molecules in the vascular endothelium [9]. The concentration of VEGF during retinal vein occlusion in the aqueous humor correlates with the severity of macular edema, iris neovascularization, and vascular permeability [10].

Pericyte administration after establishment of retinal pathology was associated with relative stabilization of OCT findings at the earlier observation point; however, retinal abnormalities remained detectable at the end of the observation

period. Given the small number of animals and the spontaneous regression observed in untreated rabbits, further studies with larger groups and quantitative retinal assessments are required to determine the therapeutic effect of pericytes. Overall, repeated intravitreal administration of VEGF and bFGF provides a relatively simple approach for inducing experimental retinal vascular pathology without implantation of a sustained-release polymer.

CONCLUSION

Intravitreal administration of VEGF and bFGF induced retinopathy-like abnormalities in rabbits. Three administrations of 400 ng of each factor were sufficient to produce retinal changes including vascular dilation and tortuosity, microaneurysms, exudative changes, and intraretinal microvascular abnormalities.

AUTHOR CONTRIBUTIONS

Conceptualization, I.A., Methodology, I.A.; Investigation, I.A., D.A., S.M. (Shakhatbayev Miras), A.C., B.M., and U.Z.; validation, K.E. and U.Z.; formal analysis, I.A.; data curation, I.A. and U.Z.; writing original draft preparation, I.A.; writing and editing, I.A. and S.M. (Sarsenova Madina); visualization, I.A., D.A. and A.C.; supervision, I.A.; project administration, I.A. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant number AP23490103 «The study of the therapeutic potential of adipose tissue pericytes in a diabetic retinopathy model».

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УДК 573.6

**VEGF ЖӘНЕ bFGF-ТІ ҚАЙТАЛАП ИНТРАВИТРЕАЛЬДІ ЕНГІЗУ АРҚЫЛЫ ҚОЯНДАРДА
ЭКСПЕРИМЕНТТІК РЕТИНОПАТИЯ МОДЕЛІН АЛУ**

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АНДАТПА

Ретинопатия – еңбекке қабілетті халық арасында көру қабілетінің ерте төмендеу себептерінің бірі. Ретинопатия қант диабетінің жиі кездесетін асқынуы болып табылады. Алайда диабеттік ретинопатияның жануарлар модельдерінде жаңа емдеу әдістерін клиникаға дейінгі зерттеу симптомдардың ұзақ уақыттан кейін пайда болуына және жануарларды ұстауға кететін жоғары шығындарға байланысты күрделі болып қала береді. Шешімдердің бірі – қант диабетін индукцияламай, ретинопатияға ұқсас модельдерді жасау. Мұндай модельдерді алудың тәсілдерінің бірі – қан тамырлары эндотелийінің өсу факторы және негізгі фибробласттар өсу факторы сияқты факторларды қолдану арқылы ретинопатияны туындату. Біздің зерттеуімізде осы факторлардың төмен концентрациялардағы комбинациясының тұрақты ретинопатия моделін индукциялау мүмкіндігі зерттелді. Нәтижелер факторлардың концентрациясы төмендетілгеніне қарамастан, оларды қайталап енгізу аурудың барлық негізгі белгілерін көрсететін модельдің қалыптасуына әкелгенін көрсетті.

Түйінді сөздер: ретинопатия, қоян моделі, VEGF, bFGF, оптикалық когерентті томография.

УДК 573.6

**ПОЛУЧЕНИЕ ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ РЕТИНОПАТИИ У КРОЛИКОВ С
ИСПОЛЬЗОВАНИЕМ ПОВТОРНОГО ИНТРАВИТРЕАЛЬНОГО ВВЕДЕНИЯ VEGF И bFGF**

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АБСТРАКТ

Ретинопатия является одной из причин раннего снижения зрения среди трудоспособного населения. Ретинопатия – распространённое осложнение сахарного диабета. Однако доклиническое тестирование новых методов лечения на животных моделях диабетической ретинопатии остаётся сложным из-за длительного периода до появления симптомов и высоких затрат на содержание животных. Одним из решений является создание моделей, сходных с ретинопатией, без индукции сахарного диабета. Одним из подходов к созданию таких моделей является индукция ретинопатии с использованием таких факторов, как сосудистый эндотелиальный фактор роста и основной фактор роста фибробластов. В нашем исследовании мы оценивали способность комбинации этих факторов в низких концентрациях индуцировать стабильную модель ретинопатии. Результаты показали, что, несмотря на снижение концентраций факторов, их повторное введение привело к формированию модели, характеризующейся всеми основными признаками заболевания.

Ключевые слова: Ретинопатия, модель на кроликах, VEGF, bFGF, оптическая когерентная томография.