Heliyon 10 (2024) e37416

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The potential of brimonidine for myopia treatment: Targeting MMP-2 to regulate choroidal thickness and control eye growth

Aiqun Xiang [a], Zixuan Peng [b], Hong He [b], Xuyun Meng [a], Yanting Luo [b], Junming Yang [b], Fang Zeng [a], Xiaolian Chen [b], Xingwu Zhong [a,b,*]

[a] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangzhou, China
[b] Hainan Eye Hospital and Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Haikou, China

A B S T R A C T

Drug treatment studies are a focal point for identifying novel approaches to reduce myopia progression through basic science research. Here, we investigated the effects of various brimonidine administration routes and concentrations on form-deprivation myopia (FDM) progression, matrix metalloproteinase-2 (MMP-2), and collagen alpha1 chain of type I (COL1A1) expression in the retinal pigment epithelial (RPE)-choroid complex and sclera of guinea pigs. They demonstrate that brimonidine has the capacity to impede choroidal thinning induced by FDM, potentially through the induction of choroidal vasodilation. Additionally, we observed that brimonidine effectively counteracts FDM-induced downregulation of choroidal and scleral MMP-2 expression. Suppression of MMP-2 expression may reduce disruption of scleral and choroidal structural integrity which reduces declines in choroidal blood circulation and mitigates increases in ocular elongation. This research elucidates the effects of brimonidine on myopia progression, offering potential insights into therapeutic interventions for myopia.

1. Introduction

Myopia has emerged as a global public health concern. Projections indicate that by 2050, approximately 5 billion individuals will be affected by myopia, with 1 billion (equivalent to one-tenth of the world's population) experiencing high myopia [1]. At present, there is no definitive cure for myopia. Although increased outdoor activity can mitigate myopia incidence, controlling the conditions proves challenging. Approaches such as orthokeratology lenses, soft bifocals, and multifocal contact lenses have shown the potential to inhibit myopia progression [2]. However, widespread adoption faces obstacles due to their high cost, low compliance, and the risk of corneal infection. Consequently, drug treatment of this condition remains a focal point for elucidating novel approaches to improve therapeutic management of myopia. Atropine, pirenzepine, and 7-methylxanthine, among others, have demonstrated efficacy in slowing down myopia progression [3].

It is now understood that brimonidine, an α_2 adrenoceptor agonist, reduces aqueous humor production and enhances uveoscleral drainage, leading to decreased intraocular pressure. Studies focusing on optic nerve protection have revealed brimonidine's neuroprotective properties in various optic neuropathies. This effect is achieved by increasing the expression of basic fibroblast growth factor (bFGF) or modulating N-methyl-D-aspartate receptor signaling in retinal ganglion cells, thereby attenuating excitotoxicity and improving neuron survival [4–6]. Liu et al. [7] discovered that the use of 0.1 % and 0.2 % brimonidine eye drops in guinea pigs with optical defocus myopia significantly lowered intraocular pressure in the intervention group compared to the non-intervention group,

* Corresponding author. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangzhou, China.
E-mail address: zhongxwu@mail.sysu.edu.cn (X. Zhong).

https://doi.org/10.1016/j.heliyon.2024.e37416
Received 13 February 2024; Received in revised form 22 August 2024; Accepted 3 September 2024
Available online 4 September 2024

effectively inhibiting axial growth. Building upon previous research, Carr et al. [8] reported that intravitreal injection of high concentrations of brimonidine can inhibit the progression of form-deprivation myopia in chickens.

Myopia is primarily caused by the excessive elongation of the eyeball, as confirmed in the form-deprivation myopia (FDM) animal model [9]. Researchers have successfully developed the FDM model in various animals, such as tree shrews, monkeys, chickens, guinea pigs, and mice [9]. This model has been utilized to investigate the pathogenesis of myopia. The potential interplay between matrix metalloproteinase-2 (MMP-2) and collagen alpha1 chain of type I (COL1A1) may play a crucial role in the progression of myopia. MMP-2, known for its involvement in matrix remodeling and collagen-decomposing activity [10,11], becomes a significant factor during myopia development. Increases in its activity during myopia development account for rises in the breakdown of COL1A1 and rendering the eyeball more prone to elongation. This susceptibility promotes elongation of the eye axis, thereby accelerating the progression of myopia [12,13].

In our preliminary research, we observed that intravitreal injection of 20 μg (4 μg/μl) brimonidine effectively inhibited the progression of FDM in guinea pigs. Concurrently, the expression of *Mmp2* and *Colla1* genes in the retina was upregulated [14]. However, it remains uncertain whether brimonidine can modulate the expression of MMP-2 and COL1A1 in the choroid and sclera. This study entailed evaluation of the dose dependent effects of brimonidine on the sclera and choroid during FDM in guinea pigs.

2. Results

2.1. Effects of brimonidine on FDM in Guinea pigs under different administration routes and concentrations

The refraction, axial length data, and the pairwise comparison between treated eyes and fellow eyes are presented in Supplementary Table S1 and Table S2. Prior to the experimental intervention, baseline measurements across all groups exhibited no statistical significance (All $P < 0.05$). To simplify the results, we exclusively compared all administration treatment groups with the FDM group.

Within the eye drop groups, results indicated that on day 20, the interocular difference in refraction in the 400 μg group was

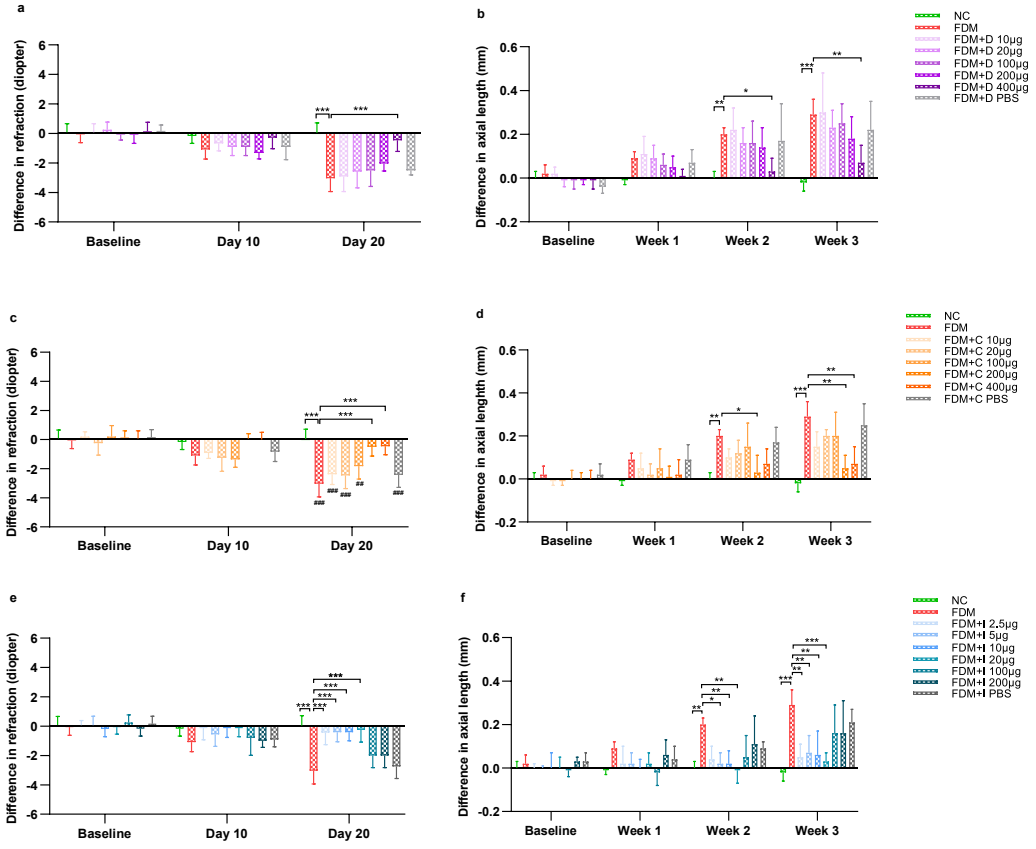

Fig. 1. Effects of brimonidine on FDM in guinea pigs under different administration routes and concentrations. a–b: the interocular differences of refraction (a) and AL (b) in eye drops groups. c–d: the interocular differences of refraction (c) and AL (d) in subconjunctival injection groups. e–f: the interocular differences of refraction (c) and AL (d) in intravitreal injection groups. NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection, PBS: phosphate-buffered saline. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

significantly lower than the FDM group ($P < 0.001$, Fig. 1a). Similarly, the interocular difference in axial length measurements in the 400 μg group was demonstrably lower than those in the FDM group at both week 2 ($P = 0.049$) and week 3 ($P = 0.005$, Fig. 1b).

In the subconjunctival injection groups, the interocular difference in refraction in the 200 μg ($P < 0.001$) and 400 μg ($P < 0.001$) groups was lower than the FDM group on day 20 (Fig. 1c). Additionally, the interocular difference in AL in the 200 μg group at week 2 ($P = 0.039$) and week 3 ($P = 0.001$), and in the 400 μg group at week 3 ($P = 0.008$) was less than the FDM group (Fig. 1d).

Eyes treated with intravitreal injection at doses of 2.5 μg, 5 μg, 10 μg, and 20 μg exhibited lower refraction differences than the FDM group on day 20 (All $P < 0.001$, Fig. 1e). At week 2, the AL difference in the 5 μg ($P = 0.011$), 10 μg ($P = 0.009$), and 20 μg ($P = 0.001$) groups was less than the FDM group. Furthermore, the AL difference in all these four groups was less than the FDM group at week 3 (All $P < 0.01$, Fig. 1f).

During the investigation of different administration methods and concentrations of brimonidine on FDM in guinea pigs, it was observed that the eye drop 400 μg group, subconjunctival injection 200 μg and 400 μg groups, and intravitreal injection 2.5 μg, 5 μg, 10 μg, 20 μg groups were associated with significantly slowed progression of refraction and AL. However, no significant differences in refraction and AL were noted between the effective treatment groups (Fig. 2).

2.2. Brimonidine can increase choroidal thickness (ChT) and choroidal blood flow in guinea pigs

We utilized optical coherence tomography angiography (OCTA) to assess changes in ChT and choroidal blood flow in the NC group, FDM group, and the brimonidine effective treatment groups. Results revealed a significant decrease in ChT in the FDM group at weeks 2 ($P = 0.004$) and 3 ($P < 0.001$). Brimonidine effectively inhibited this decrease at week 3, with the ChT in the subconjunctival injection 400 μg group ($P = 0.044$) and intravitreal injection 5 μg ($P = 0.019$), 10 μg ($P = 0.007$), and 20 μg ($P = 0.001$) groups being significantly higher than the FDM group (Fig. 3b).

For the choroidal blood flow parameters, changes in the vessel perimeter index (VPI) and blood flow signal intensity (flux) were investigated in the choriocapillaris layer and medium-large vessel layers. In the choriocapillaris layer of the FDM group, VPI and flux exhibited a decreasing trend. The VPI of the FDM group was significantly less than the NC group at weeks 2 ($P = 0.006$) and 3 ($P = 0.001$). Brimonidine treatment ameliorated this phenomenon, as the VPI of the intravitreal injection 5 and 10 μg groups was larger than the FDM group at weeks 2 ($P < 0.001$, $P = 0.005$, respectively) and 3 (All $P < 0.001$, Fig. 4a). The VPI of the 2.5 μg group was also larger than the FDM group at week 3 ($P = 0.017$, Fig. 4a). However, brimonidine intervention did not alter flux in the choriocapillaris layer. At weeks 2 and 3, the flux of the FDM group and the brimonidine-effective treatment groups were less than the NC group (All $P < 0.05$, Fig. 4b). In the medium-large vessel layer, the VPI in the FDM group was also less than the NC group at week 3 ($P = 0.001$). Simultaneously, the VPI in the subconjunctival injection 200 μg ($P = 0.006$), 400 μg ($P = 0.044$), intravitreal injection 2.5 μg ($P = 0.004$), 5 μg ($P = 0.031$), 10 μg ($P = 0.008$), and 20 μg ($P = 0.001$) groups were larger than the FDM group (Fig. 4c). However, there was no statistical significance in the flux comparison between these groups in the medium-large vessel layers (All $P > 0.05$, Fig. 4d).

2.3. Brimonidine upregulated MMP-2 expression in the RPE-choroid complex of myopic eyes

In our investigation, we further examined the expression of MMP-2 and COL1A1 in the RPE-choroid complex and sclera within the NC group, FDM group, and brimonidine effective treatment groups using both immunofluorescence and Western blot analyses.

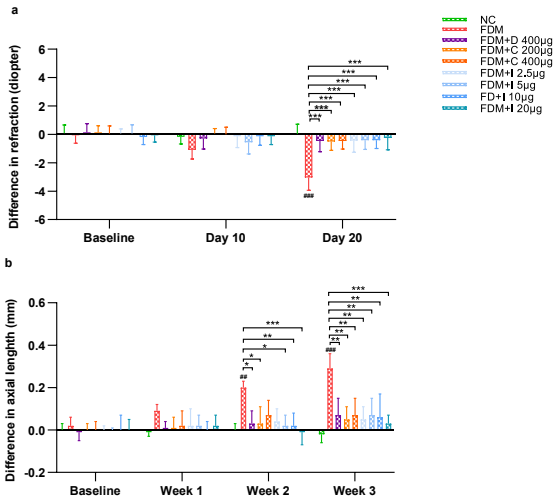

Fig. 2. Comparison of interocular differences of refraction (a) and AL (b) in the effective treatment groups. NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. #: vs. NC $P < 0.05$, ##: vs. NC $P < 0.01$, ###: vs. NC $P < 0.001$.

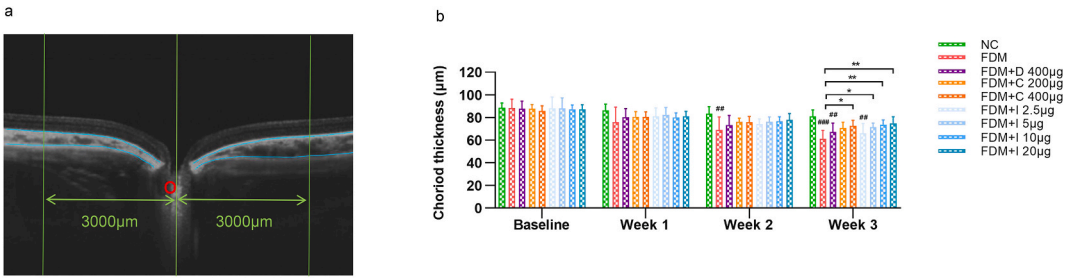

Fig. 3. OCT image (a) and comparison of ChT in the effective treatment groups (b). O: optic nerve, NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. #: vs. NC $P < 0.05$, ##: vs. NC $P < 0.01$, ###: vs. NC $P < 0.001$.

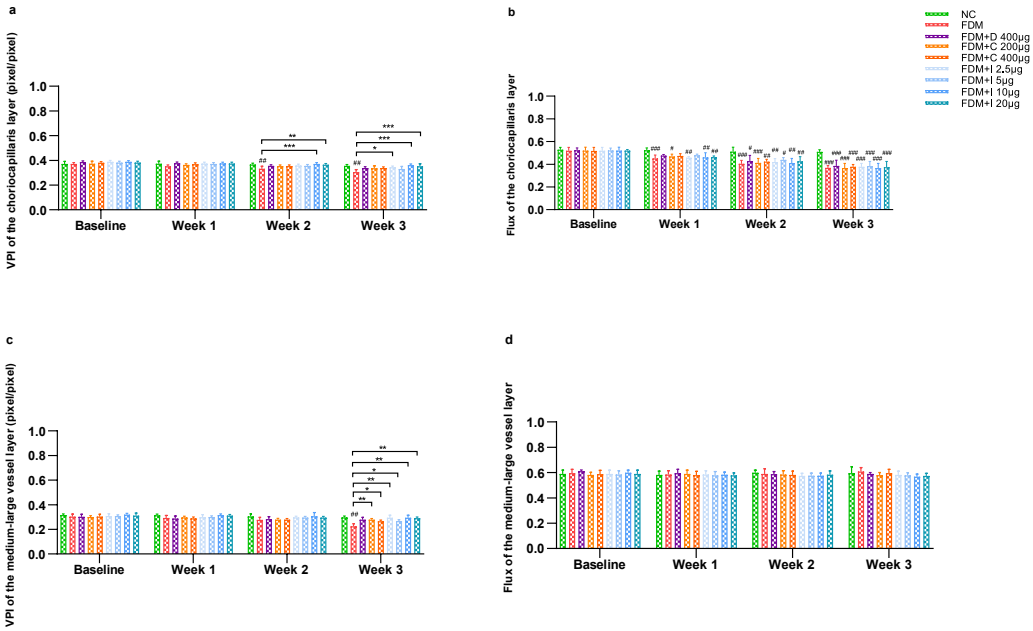

Fig. 4. Comparison of the choroidal VPI and flux in the effective treatment groups. a: VPI of the choriocapillaris layer, b: flux of the choriocapillaris layer, c: VPI of the medium-large vessel layer, d: flux of the medium-large vessel layer. NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. #: vs. NC $P < 0.05$, ##: vs. NC $P < 0.01$, ###: vs. NC $P < 0.001$.

However, it's worth noting that COL1A1 was nearly undetectable in the RPE-choroid complex; therefore, only MMP-2 was assessed in this region. Immunofluorescence images revealed that, compared to the NC group, MMP-2 expression was upregulated, and COL1A1 expression was downregulated in the FDM group. Conversely, when compared to the FDM group, the brimonidine effective treatment groups exhibited downregulated MMP-2 expression (Fig. 5a). Consistent with immunofluorescence findings, Western blot analyses demonstrated similar trends (Fig. 5b and c). Specifically, in comparison to the NC group, MMP-2 protein expression in the RPE-choroid complex ($P < 0.001$, Fig. 5d) and sclera ($P < 0.001$, Fig. 5e) was significantly upregulated, while COL1A1 expression in the sclera ($P < 0.001$, Fig. 5f) was significantly downregulated in the FDM group. Notably, brimonidine treatment effectively inhibited the upregulation of MMP-2 in both the RPE-choroid complex and sclera of myopic eyes (All $P < 0.001$, Fig. 5d and e). However, the protein expression of COL1A1 in the sclera remained unaffected by brimonidine.

3. Discussion

In this study, our primary focus was to investigate the impact of brimonidine on the development of form-deprivation myopia in guinea pigs. The findings revealed that the effective doses for inhibiting myopia varied based on different administration methods. Notably, topical and subconjunctival injections required higher concentrations to impede myopia progression compared to intravitreal injections. The rationale behind this observation lies in the transmission of abnormal visual signals from photoreceptors to the RPE and

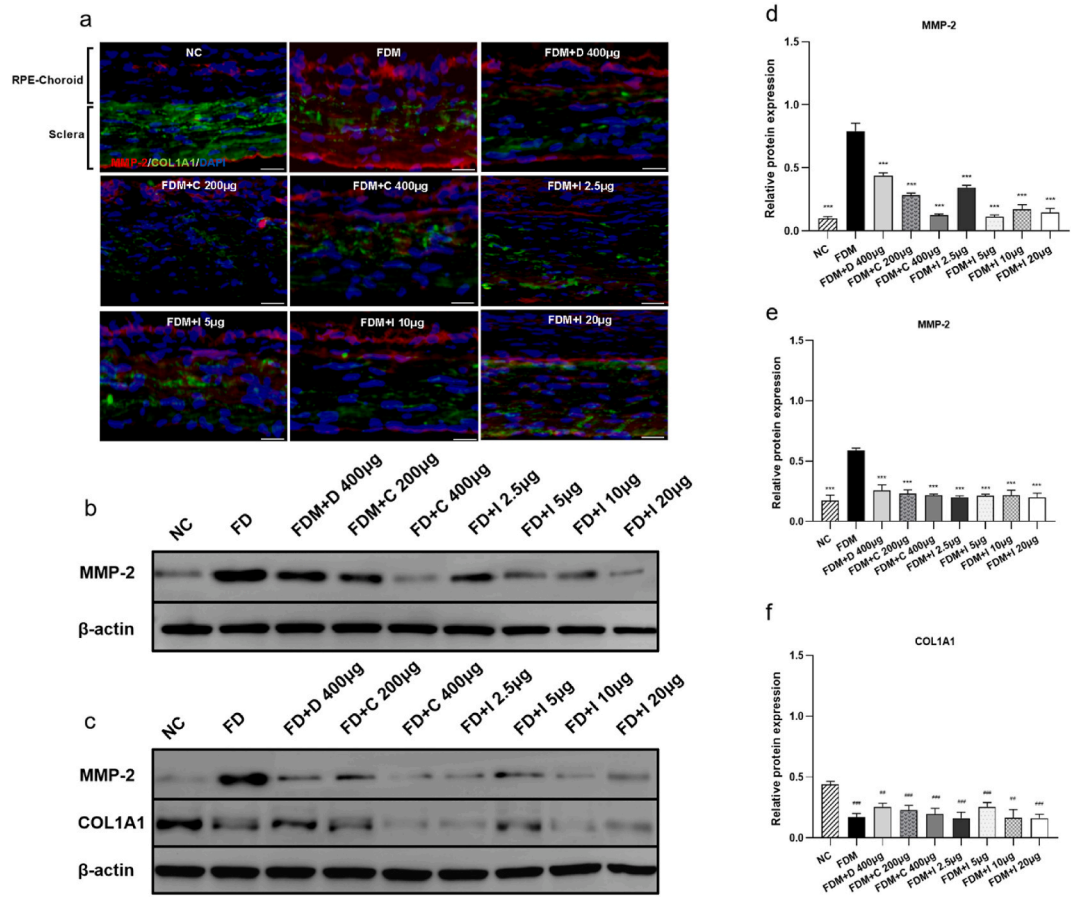

Fig. 5. Immunofluorescence and Western blot analyses of MMP-2 and COL1A1 expression in the RPE-choroid complex and sclera in the effective treatment groups (bar = 50 μm). a: immunofluorescence images of MMP-2 and COL1A1 expression, b: Western blot result of MMP-2 expression in the RPE-choroid complex, c: Western blot results of MMP-2 and COL1A1 expression in the sclera. d: relative protein expression of MMP-2 in the RPE-choroid complex, e–f: relative protein expression of MMP-2 (e) and COL1A1 (f) in the sclera. NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection. *: vs. FDM $P < 0.05$, **: vs. FDM $P < 0.01$, ***: vs. FDM $P < 0.001$. #: vs. NC $P < 0.05$, ##: vs. NC $P < 0.01$, ###: vs. NC $P < 0.001$.

subsequently to the choroid, ultimately triggering remodeling of the scleral extracellular matrix (ECM) [15]. Consequently, drug research for myopia prevention and treatment predominantly targets the posterior segment of the eye. Intravitreal injection stands out as a method that allows direct drug entry into the retina, subsequently acting on the choroid and sclera. This direct drug effect on the target site is a significant advantage. A previous study from our research group consistently demonstrated that intravitreal injection of low-dose brimonidine could maintain elevated drug concentrations in the retina and sclera [16]. Conversely, topical administration and subconjunctival injections face challenges related to reduced drug bioavailability attributed to biological barriers such as the cornea and sclera, ocular drainage, and absorption into the systemic circulation [17,18]. As a result, higher drug concentrations are required for topical administration and subconjunctival injections to effectively inhibit the progression of myopia.

This study appears not to be dose dependent which agrees with other studies describing three different modes of action of brimonidine. In our earlier study, we posited that at low concentrations, the drug might not reach the threshold for a significant therapeutic effect, while at high concentrations, saturation of the receptors or targets it interacts with may occur [14]. This theoretical framework is in line with the current findings. However, the precise mechanism through which brimonidine hinders the progression of myopia, particularly its interaction with α_2 receptors, remains unclear. Carr et al. [19] reported that muscarinic antagonists could block α_2-adrenoceptor agonists at concentrations comparable to those inhibiting myopia in chicks *in vivo*. This led to the hypothesis that atropine-like muscarinic antagonists could inhibit myopia via α_2-adrenergic receptors. Similarly, it is very conceivable that brimonidine's inhibition of myopia progression involves off-target drug binding, as the drug concentration in the retina and sclera far exceeds the level required to activate α_2 receptors after intravitreal injection of 20 μg (4 μg/ul) brimonidine [16,20].

The advancement of OCTA technology has brought attention to the evolving understanding of changes in ChT and choroidal blood flow in the context of myopia development. Numerous animal experiments [21–23] and clinical studies [24–26] have consistently demonstrated a significant correlation between choroidal thinning and the degree of refractive error. Additionally, myopic eyes typically exhibit a reduction in choroidal vessel thickness and blood perfusion [27–29]. Herein, we investigated whether brimonidine

could suppress FDM-induced choroidal thinning and declines in blood perfusion.

Our findings that FDM induced choroidal thinning in guinea pigs agree with those reported in other studies [28–30]. In our analysis of choroidal blood flow parameters, we observed a reduction in the VPI in both the choriocapillaris layer and the medium-large vessel layer, while flux solely decreased in the choriocapillaris layer. This observation agrees with a report by Yu et al. [22], who reported a significant decrease in blood vessel density in the choriocapillaris layer in lens-induced myopia guinea pigs, while the change in blood vessel density in the medium-large vessel layer was not statistically significant in previous studies. These results suggest variations in the regulation of myopia signal stimulation by different vascular layers. The choriocapillaris layer, being in close proximity to the retina, appears to be more sensitive to various physiological changes induced by myopia. On the other hand, the medium to large vessel layer may possess a more robust regulatory mechanism. Even though the VPI decreased, blood flow remained relatively adequate to support tissue function. The intricate mechanisms underlying these observations may necessitate further in-depth studies and experiments for comprehensive elucidation.

Our observations indicate that brimonidine can effectively counter choroidal thinning induced by FDM and ameliorate the reduction in VPI in the choriocapillaris layer as well as the medium-large vessel layer. Previous reports have highlighted that peribulbar injection of prazosin [29] and topical application of bunazosin [30] (alpha-adrenergic antagonist) can enhance choroidal perfusion. Interestingly, some α_2-adrenoceptor agonists have been demonstrated to induce vasodilation [31,32]. Brimonidine, as a highly selective α_2-adrenergic receptor agonist, has been shown to enhance blood flow to the eyes [33,34]. Moreover, brimonidine can induce a modest vasodilatory response in certain vessels by activating α_2-adrenergic receptors and subsequently producing nitric oxide (NO) [35]. The increase in VPI observed following brimonidine treatment may be indicative of its vasodilatory effect, potentially contributing to choroidal thickening. Notably, brimonidine did not exert an impact on blood flow signal intensity. This parameter is linked to the number of red blood cell particles passing through per unit of time. Therefore, even if blood vessel dilation increases, without significant changes in blood flow velocity, the blood flow signal remains relatively stable.

Both experimental and clinical evidence substantiate that excessive elongation of the eyeball in myopia is linked to alterations in the scleral ECM. The sclera is primarily comprised of type I collagen [36], and MMP-2 plays a role in initiating the degradation of scleral collagen [37]. Numerous studies have reported an increase in MMP-2 expression and a decrease in COL1A1 expression in the sclera of myopic animals [38–42], a pattern we also observed in FDM guinea pigs. Previous research has suggested that brimonidine can upregulate neurotrophic factors like basic fibroblast growth factor (bFGF) [43], and bFGF can inhibit the production of MMP-2 [44]. Consistent with these findings, we observed that brimonidine led to the downregulation of MMP-2, subsequently inhibiting the progression of myopia. The choroid, known to synthesize MMP-2, interacts with neighboring cells through receptor-mediated effects [11,45]. Consequently, MMP-2 is not only involved in the systemic maintenance of choroidal vessels but may also exert effects on scleral tissue. By inhibiting the activity of MMP-2, brimonidine could potentially influence the thickness and structural stability of both the choroid and sclera, thereby regulating choroidal blood supply and the growth of the eyeball.

3.1. Limitations of the study

While our study provides valuable insights, it is important to acknowledge certain limitations. Firstly, the guinea pig, although a widely used animal model for myopia, lacks retinal blood vessels. This feature allows for effective study of changes in choroidal blood

Table 1
Experimental group design.

Groups	Drug concentrations	Sample size, n
NC	–	6
FDM	–	8
FDM + D 10 μg	2 μg/μl	6
FDM + D 20 μg	4 μg/μl	6
FDM + D 100 μg	20 μg/μl	6
FDM + D 200 μg	40 μg/μl	6
FDM + D 400 μg	80 μg/μl	7
FDM + D PBS	–	6
FDM + C 10 μg	2 μg/μl	6
FDM + C 20 μg	4 μg/μl	6
FDM + C 100 μg	20 μg/μl	7
FDM + C 200 μg	40 μg/μl	7
FDM + C 400 μg	80 μg/μl	7
FDM + C PBS	–	6
FDM + I 2.5 μg	0.5 μg/μl	8
FDM + I 5 μg	1 μg/μl	8
FDM + I 10 μg	2 μg/μl	8
FDM + I 20 μg	4 μg/μl	8
FDM + I 100 μg	20 μg/μl	6
FDM + I 200 μg	40 μg/μl	6
FDM + I PBS	–	6

NC: normal control, FDM: form-deprivation myopia, D: eye drops, C: subconjunctival injection, I: intravitreal injection, PBS: phosphate-buffered saline.

flow but introduces structural differences compared to the human eye. Future research could consider exploring non-primate mammal models to enhance the translatability of findings. Secondly, the manual division of choroidal layers in our study may introduce some errors. The field could benefit from the development of more comprehensive and automated OCTA instruments in the future, potentially minimizing such inaccuracies. Lastly, poor drug solubility limited the concentration range over which drug effects could be evaluated. In subsequent research, considering alternative solvents, may provide an avenue for exploring higher concentrations of brimonidine and and more extensively elucidating other dose response relationships.

4. Materials and methods

The experiment was conducted in three parts, adhering to protocols approved by the Medical Ethics Review Committee of Hainan Eye Hospital (2022-039-01) and following the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three-week-old male tricolor guinea pigs were randomly assigned to groups and housed in a controlled environment of 25 °C temperature, 300 lux illumination, and a 12-h light/dark cycle with unrestricted access to food, water, and fresh vegetables.

1) Experiment 1 aimed to identify effective administration routes and concentrations of brimonidine for mitigating FDM in guinea pigs. Various doses of brimonidine tartrate (10–200 μg) were administered via eye drops, subconjunctival injections, and intravitreal injections to different groups according to the data we published [46]. Based on results and brimonidine solubility, additional groups received higher doses: 400 μg eye drops, 400 μg subconjunctival injection, and 2.5/5 μg intravitreal injections. Grouping details are provided in Table 1. Eye drops were administered twice daily (12 h apart), while injections occurred every 96 h, following established protocols. Refraction and axial length changes were monitored in each group.
2) Experiment 2 investigated the impacts of brimonidine on ChT and choroidal blood flow in guinea pigs with FDM. Employing non-invasive OCTA, ChT, choroidal vessel perimeter index, and blood flow signal intensity were measured in various groups: normal control group, FDM group, and brimonidine-treated groups receiving: 400 μg eye drops 200 μg and 400 μg subconjunctival injections, and intravitreal injections of 2.5 μg, 5 μg, 10 μg, and 20 μg. This experiment sought to elucidate the physiological changes associated with brimonidine's effectiveness in mitigating FDM.
3) The third experiment explored the potential link between brimonidine's ability to inhibit FDM progression and reduce declines in structural stability of the choroid and sclera in guinea pigs. This investigation involved analyzing the expression of key proteins, namely MMP-2 and COL1A1, in the RPE-choroid complex and the sclera.

4.1. Animal model

To induce form-deprivation, a custom opaque balloon apparatus was employed. The initial design consisted of a cropped 8-inch balloon secured over the right eye of each guinea pig. As the animals grew, this was replaced with a 10-inch balloon to maintain consistent coverage. Notably, all interventions were restricted to the right eye, while the left eye served as an untreated control. For the normal control group, both eyes remained untouched throughout the experiment.

4.2. Drug preparation and administration

To prepare brimonidine tartrate solutions, the desired amount was dissolved in phosphate-buffered saline. For eye drops, 5 μl were administered twice daily. Prior to subconjunctival and intravitreal injections, topical anesthesia with proparacaine hydrochloride was applied. Both injections utilized a 5 μl Hamilton microsyringe, with a 30-gauge needle for subconjunctival and a 33-gauge needle for intravitreal administration. 5 μl of solution was injected into the right eye of each animal. More details on the intravitreal injection procedure are provided in our previous work [16,46].

5. Biometric parameters measurement

Refraction diopters were determined by an experienced optometrist in a darkroom using a handheld fringe retinoscope. Prior to examination, 0.5 % compound tropicamide eye drops were administered to paralyze the ciliary muscle. Spherical equivalent measurements were averaged from three repetitions per eye. Axial length (AL) was assessed via A-scan ultrasonography (AXIS II, Quantel Medical, France) after anesthetizing the eyes with proparacaine hydrochloride drops. Ultrasound velocities specific to the anterior chamber (1557.5 m/s), lens (1723.3 m/s), and vitreous cavity (1540 m/s) were employed. Each eye's AL was measured five times, and the average was recorded.

5.1. Sample collection

Following the completion of the experimental procedures, all guinea pigs were euthanized humanely through intraperitoneal injection of an overdose of pentobarbital. The eyeballs were then carefully excised for further analysis. For immunofluorescence studies, the extracted eyeballs were promptly immersed in FAS eyeball fixative solution to preserve their cellular structures. Following a thorough dehydration process, the eyeballs were embedded in OCT compound, a cryoprotectant medium, and subsequently frozen for sectioning into thin slices. For Western blot, the cornea, lens, vitreous body, and retina were removed, the RPE-choroid complex

and the sclera were collected, then quick-frozen in dry ice and stored at −80 °C until analysis.

5.2. Measurement of ChT and choroidal blood flow

ChT and choroidal blood flow parameters were assessed using an animal-specific optical coherence tomography/angiography system (OCT/OCTA, Optoprobe, UK). Prior to the examination, 0.5 % compound tropicamide eye drops were administered to induce pupil dilation, followed by anesthesia using 1.5 % sodium pentobarbital (37.5 mg/kg). Scans were performed with the optic disc as the center to acquire structural OCT (Fig. 3a) and OCTA (Fig. S1) images. For ChT measurement, the structural OCT image passing through the optic nerve was selected. The choroidal boundary was delineated continuously at 3000 μm on both sides of the optic nerve, and the built-in software automatically calculated the ChT. The boundary definition for the choriocapillaris layer and the medium-large vessel layer followed established protocols [22,47] with manual corrections based on vascular morphology. The imaging system processed blood vessels into binary black-and-white images, subsequently employing them to calculate the VPI. The binary image brightness percentage parameter was fixed to 0.3. Blood flow signal intensity, reflecting the number of particles traversing the imaging plane per unit time, was normalized to a dynamic range of 0–1. Throughout this study, we refer to normalized blood flow signal intensity as "flux".

6. Immunofluorescence

Frozen sections were allowed to thaw at room temperature for 30 min, immersed in PBS for 10 min and fixed using 4 % paraformaldehyde for 15 min. Following three rinses with PBS, the sections were permeated with 0.3 % Triton-X 100 for 10 min and subjected to another PBS wash. Subsequently, the sections were blocked with 0.5 % bovine serum albumin for 30 min and then incubated overnight at 4 °C with mouse anti-MMP-2 (Novus Biologicals, NB200-113, 1:50) and goat anti-Collagen I antibody (arigo Biolaboratories Corp, ARG21965, 1:500). After PBS washing, the sections were exposed to secondary antibodies: chicken anti-goat IgG conjugated to Alexa Fluor 488 and donkey anti-mouse IgG conjugated to Alexa Fluor 594 for 2 h at room temperature. Following the removal of excess secondary antibody with PBS, 0.5 μg/ml DAPI was added for 5 min. The sections were then observed using an inverted fluorescence microscope (Olympus-U-HGLGPS).

6.1. Western blot

To prepare the tissue samples, RIPA lysis buffer (Servicebio) and protease inhibitors were added, followed by homogenization. The lysate was then centrifuged at 12,000 rpm, 4 °C, for 10 min. Protein concentration was determined using the BCA kit. Subsequently, protein extracts were loaded onto 5 % SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. The transferred membranes underwent washing and blocking with TBST and 5 % skim milk for 30 min at room temperature. They were then incubated overnight at 4 °C with mouse anti-MMP-2 (Proteintech, 10373-2-AP, 1:1000) and goat anti-Collagen I antibody (arigo Biolaboratories Corp, ARG21965, 1:1000). Following this, the membranes were incubated with horseradish peroxidase (HRP)-conjugated donkey anti-goat and HRP-goat anti-rabbit secondary antibodies for 30 min at room temperature. The bands were visualized using chemiluminescence kits and detected with a chemiluminescence instrument (CLINX 6100). Image J software was employed for image analysis.

6.2. Statistical analysis

Statistical analysis was performed using SPSS software (Version 26.0). Descriptive statistics are presented as mean ± SD. Group differences were assessed through one-way analysis of variance (One-way ANOVA). Post-tests were conducted using the Bonferroni method in cases of homogeneity of variance and Dunnett's T3 if homogeneity was not satisfied. A paired sample *t*-test was employed to compare differences between treated eyes and fellow eyes. Statistical significance was set at a P value < 0.05.

Funding

This work was supported by grants from the National Natural Science Foundation of China (82271116), Hainan Province Clinical Medical Center, and Science and Technology Planning Project of Hainan Province (ZDYF2022SHFZ326, LCYX202406).

Data availability statement

Data will be made available on request from the corresponding author.

CRediT authorship contribution statement

Aiqun Xiang: Writing – original draft, Investigation, Data curation. **Zixuan Peng:** Investigation, Data curation. **Hong He:** Methodology. **Xuyun Meng:** Writing – original draft, Formal analysis. **Yanting Luo:** Investigation, Data curation. **Junming Yang:** Investigation, Formal analysis. **Fang Zeng:** Writing – review & editing, Formal analysis. **Xiaolian Chen:** Supervision, Resources. **Xingwu Zhong:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge J.W. and his colleagues from Beijing HealthOLight Technology Co., Ltd. (Beijing, China) for technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37416.

References

[1] B.A. Holden, T.R. Fricke, D.A. Wilson, et al., Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050 [J], Ophthalmology 123 (5) (2016) 1036–1042.
[2] J.B. Jonas, M. Ang, P. Cho, et al., IMI prevention of myopia and its progression [J], Investigative Opthalmology & Visual Science 62 (5) (2021) 6.
[3] P. Ganesan, C.F. Wildsoet, Pharmaceutical intervention for myopia control [J], Expet Rev. Ophthalmol. 5 (6) (2010) 759–787.
[4] R. Wen, T. Cheng, Y. Li, et al., α_2-Adrenergic agonists induce basic fibroblast growth factor expression in Photoreceptors**In vivo**and ameliorate light damage [J], J. Neurosci. 16 (19) (1996) 5986–5992.
[5] E. Yoles, L. Wheeler, M. Schwartz, Alpha2-adrenoreceptor agonists are neuroprotective in a rat model of optic nerve degeneration. [J], Invest. Ophthalmol. Vis. Sci. (1999).
[6] E. Woldemussie, G. Ruiz, M. Wijono, et al., Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. [J], Invest. Ophthalmol. Vis. Sci. (2001).
[7] Y. Liu, Y. Wang, H. Lv, et al., α-adrenergic agonist brimonidine control of experimentally induced myopia in Guinea pigs: a pilot study [J], Mol. Vis. (2017).
[8] B.J. Carr, C.T. Nguyen, W.K. Stell, Alpha 2 -adrenoceptor agonists inhibit form-deprivation myopia in the chick [J], Clin. Exp. Optom. 102 (4) (2019) 418–425.
[9] D. Troilo, E.L. Smith, D.L. Nickla, et al., Imi – report on experimental models of emmetropization and myopia [J], Investigative Opthalmology & Visual Science 60 (3) (2019) M31.
[10] F. Zhao, Q. Zhou, P.S. Reinach, et al., Cause and effect relationship between changes in scleral matrix metallopeptidase-2 expression and myopia development in mice [J], Am. J. Pathol. 188 (8) (2018) 1754–1767.
[11] A.R. Harper, J.A. Summers, The dynamic sclera: extracellular matrix remodeling in normal ocular growth and myopia development [J], Exp. Eye Res. 133 (2015) 100–111.
[12] J. Wang, J. Cui, H. Zhu, Suppression of type I collagen in human scleral fibroblasts treated with extremely low-frequency electromagnetic fields [J], Mol. Vis. (2013).
[13] J. Hu, D. Cui, X. Yang, et al., Bone morphogenetic protein-2: a potential regulator in scleral remodeling [J], Mol. Vis. (2008).
[14] Y. Yang, J. Wu, D. Wu, et al., Intravitreal brimonidine inhibits form-deprivation myopia in Guinea pigs [J], Eye and Vision 8 (1) (2021).
[15] W.-Y. Wang, C. Chen, J. Chang, et al., Pharmacotherapeutic candidates for myopia: a review [J], Biomed. Pharmacother. 133 (2021) 111092.
[16] A. Xiang, H. He, H. Yu, et al., Ocular posterior segment distribution and pharmacokinetics of brimonidine after intravitreal administration in Guinea pigs [J], J. Ocul. Pharmacol. Therapeut. 39 (7) (2023) 456–462.
[17] J. Barar, A.R. Javadzadeh, Y. Omidi, Ocular novel drug delivery: impacts of membranes and barriers [J], Expet Opin. Drug Deliv. 5 (5) (2008) 567–581.
[18] G.A. Rodrigues, D. Lutz, J. Shen, et al., Topical drug delivery to the posterior segment of the eye: addressing the challenge of preclinical to clinical translation [J], Pharmaceut. Res. 35 (12) (2018).
[19] B.J. Carr, K. Mihara, R. Ramachandran, et al., Myopia-inhibiting concentrations of muscarinic receptor antagonists block activation of Alpha2A-adrenoceptors in vitro [J], Investigative Opthalmology & Visual Science 59 (7) (2018) 2778.
[20] Y. Takamura, T. Tomomatsu, T. Matsumura, et al., Vitreous and aqueous concentrations of brimonidine following topical application of brimonidine tartrate 0.1% ophthalmic solution in humans [J], J. Ocul. Pharmacol. Therapeut. 31 (5) (2015) 282–285.
[21] Y. Huang, Y. Wang, Y. Shen, et al., Defocus-induced spatial changes in choroidal thickness of chicks observed by wide-field swept-source OCT [J], Exp. Eye Res. 233 (2023) 109564.
[22] T. Yu, X. Xie, H. Wei, et al., Choroidal changes in lens-induced myopia in Guinea pigs [J], Microvasc. Res. 138 (2021) 104213.
[23] W. Chen, L. Li, Q. Feng, et al., Quantitative assessment of the choroidal vessel diameter during the recovery of form-deprivation myopia in Guinea pigs [J], Curr. Eye Res. 47 (9) (2022) 1329–1338.
[24] S.A. Read, M.J. Collins, S.J. Vincent, et al., Choroidal thickness in myopic and nonmyopic children assessed with enhanced depth imaging optical coherence tomography [J], Investigative Opthalmology & Visual Science 54 (12) (2013) 7578.
[25] S.A. Read, J.A. Fuss, S.J. Vincent, et al., Choroidal changes in human myopia: insights from optical coherence tomography imaging [J], Clin. Exp. Optom. 102 (3) (2018) 270–285.
[26] L.X. Zhou, L. Shao, L. Xu, et al., The relationship between scleral staphyloma and choroidal thinning in highly myopic eyes: the Beijing Eye Study [J], Sci. Rep. 7 (1) (2017).
[27] R.A. Alshareef, M.K. Khuthaila, M. Januwada, et al., Choroidal vascular analysis in myopic eyes: evidence of foveal medium vessel layer thinning [J], International Journal of Retina and Vitreous 3 (1) (2017).
[28] X. Zhou, S. Zhang, F. Yang, et al., Decreased choroidal blood perfusion induces myopia in Guinea pigs [J], Investigative Opthalmology & Visual Science 62 (15) (2021) 30.
[29] X. Zhou, S. Zhang, G. Zhang, et al., Increased choroidal blood perfusion can inhibit form deprivation myopia in Guinea pigs [J], Investigative Opthalmology & Visual Science 61 (13) (2020) 25.
[30] H. Jeong, D. Lee, X. Jiang, et al., Topical application of bunazosin hydrochloride suppresses myopia progression with an increase in choroidal blood perfusion [J], Investigative Opthalmology & Visual Science 64 (14) (2023) 15.
[31] C. Bockman, W.B. Jeffries, P. Abel, Binding and functional characterization of alpha-2 adrenergic receptor subtypes on pig vascular endothelium. [J], J. Pharmacol. Exp. Therapeut. (1993).
[32] E. Thorin, T.-D. Nguyen, A. Bouthillier, Control of vascular tone by endogenous endothelin-1 in human pial arteries [J], Stroke 29 (1) (1998) 175–180.
[33] M. Vetrugno, Acute and chronic effects of brimonidine 0.2% on intraocular pressure and pulsatile ocular blood flow in patients with primary open-angle glaucoma: an open-label, uncontrolled, prospective study [J], Clin. Therapeut. 23 (9) (2001) 1519–1528.

[34] N. Takahashi, K. Sato, N. Kiyota, et al., The effect of a brinzolamide/brimonidine fixed combination on optic nerve head blood flow in rabbits [J], PLoS One 18 (12) (2023) e0295122.
[35] R.H. Rosa, T.W. Hein, Z. Yuan, et al., Brimonidine evokes heterogeneous vasomotor response of retinal arterioles: diminished nitric oxide-mediated vasodilation when size goes small [J], Am. J. Physiol. Heart Circ. Physiol. 291 (1) (2006) H231–H238.
[36] F.W. Keeley, J.D. Morin, S. Vesely, Characterization of collagen from normal human sclera [J], Exp. Eye Res. 39 (5) (1984) 533–542.
[37] J. Woessner, The family of matrix metalloproteinases. [J], Ann. N. Y. Acad. Sci. (1994).
[38] J. Rada, H. Brenza, Increased latent gelatinase activity in the sclera of visually deprived chicks. [J], Invest. Ophthalmol. Vis. Sci. (1995).
[39] J. Guggenheim, N. Mcbrien, Form-deprivation myopia induces activation of scleral matrix metalloproteinase-2 in tree shrew. [J], Invest. Ophthalmol. Vis. Sci. (1996).
[40] Z. Liu, F. Qiu, J. Li, et al., Adenomatous polyposis coli mutation leads to myopia development in mice [J], PLoS One 10 (10) (2015) e0141144.
[41] X. Zhou, F. Ji, J. An, et al., Experimental murine myopia induces collagen type Iα1 (COL1A1) DNA methylation and altered COL1A1 messenger RNA expression in sclera [J], Mol. Vis. (2012).
[42] M. Wang, Z.-K. Yang, H. Liu, et al., Genipin inhibits the scleral expression of miR-29 and MMP2 and promotes COL1A1 expression in myopic eyes of Guinea pigs [J], Graefe's Arch. Clin. Exp. Ophthalmol. 258 (5) (2020) 1031–1038.
[43] R.K. Lai, T. Chun, D. Hasson, et al., Alpha-2 adrenoceptor agonist protects retinal function after acute retinal ischemic injury in the rat [J], Vis. Neurosci. 19 (2) (2002) 175–185.
[44] R.D. Kenagy, C.E. Hart, W.G. Stetler-Stevenson, et al., Primate smooth muscle cell migration from aortic explants is mediated by endogenous platelet-derived growth factor and basic fibroblast growth factor acting through matrix metalloproteinases 2 and 9 [J], Circulation 96 (10) (1997) 3555–3560.
[45] B. Stéen, S. Sejersen, L. Berglin, et al., Matrix metalloproteinases and metalloproteinase inhibitors in choroidal neovascular membranes. [J], Invest. Ophthalmol. Vis. Sci. (1998).
[46] Z. Peng, A. Xiang, H. He, et al., Brimonidine as a possible treatment for myopia [J], BMC Ophthalmol. 24 (1) (2024).
[47] M. Lee, H. Lee, H.C. Kim, et al., Changes in stromal and luminal areas of the choroid in pachychoroid diseases: insights into the pathophysiology of pachychoroid diseases [J], Investigative Opthalmology & Visual Science 59 (12) (2018) 4896.