

RESEARCH ARTICLE

EVALUATION OF THE EFFECT OF INTRAVITREAL BEVACIZUMAB INJECTION ON SCLERAL THICKNESS WITH ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY

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ABSTRACT

Introduction: To evaluate the changes of scleral thickness after repeated intravitreal injections of bevacizumab in a consecutive series of patients with anterior segment optical coherence tomography (AS-OCT).

Methods: The study group consisted of 54 eyes of 54 consecutive phakic patients who were indicated for the first time for intravitreal bevacizumab injection with a follow-up time of at least 12 months. The fellow eye of each patient formed the control group. Scleral thickness at the injection side was measured before the first intravitreal injection and after re-injections using AS-OCT.

Results: Before treatment, scleral thickness was $610.5 \pm 22.9 \mu\text{m}$ at the injection site and $608.17 \pm 22.2 \mu\text{m}$ at the same quadrant in fellow eye. After treatment scleral thickness was $604.8 \pm 22.3 \mu\text{m}$ at the injection site and $607.2 \pm 19.2 \mu\text{m}$ at the same quadrant in fellow eye. There was no statistically significant change both at injection site and other quadrants in the study and fellow eye at the end of follow-up period. The mean injection number was 9.44 ± 0.76 . There was no significant correlation between the changes in scleral thickness and injection numbers ($r=0.43$, $p=0.365$).

Discussion: Well-controlled intraocular pressure after intravitreal injections and more importantly molecular weight of injected material could probably prevent scleral thinning.

Key words: intravitreal injections, sclera, optical coherence tomography, retina, bevacizumab

Intravitreal Bevacizumab Enjeksiyonunun Skleral Kalınlık Üzerine Etkisinin Ön Segment Optik Koherens Tomografi ile Değerlendirilmesi

Giriş: Tekrarlayan intravitreal bevacizumab enjeksiyonları sonrasında skleral kalınlıkta meydana gelebilecek değişimleri ön segment optik koherens tomografi ile değerlendirmek.

Yöntem: İlk kez intravitreal enjeksiyon endikasyonu alan ve en az 12 aylık takibi olan 54 fakik hastanın 54 gözü çalışma kapsamında değerlendirildi. Hastaların diğer gözleri kontrol grubunu oluşturdu. İlk enjeksiyon öncesinde ve tekrarlayan enjeksiyonlar sonrasında enjeksiyon bölgesindeki skleral kalınlık ön segment optik koherens ile değerlendirildi.

Bulgular: Tedavi öncesinde enjeksiyon bölgesinde skleral kalınlık $610.5 \pm 22.9 \mu\text{m}$, diğer gözün aynı kadranında ise $608.17 \pm 22.2 \mu\text{m}$ olarak ölçüldü. Tedavi sonrasında ise enjeksiyon bölgesinde skleral kalınlık $604.8 \pm 22.3 \mu\text{m}$, diğer gözün aynı kadranında ise $607.2 \pm 19.2 \mu\text{m}$ olarak ölçüldü. Hem enjeksiyon bölgesinde hem de diğer kadranlarda çalışma ve kontrol gözlerinde takip süresi sonunda skleral kalınlıkta istatistiksel olarak anlamlı bir değişiklik saptanmadı. Ortalama enjeksiyon sayısı 9.44 ± 0.76 idi. Enjeksiyon sayısı ile skleral kalınlıktaki değişim arasında anlamlı korelasyon bulunmadı ($r=0.43$, $p=0.365$).

Tartışma: Intravitreal enjeksiyon sonrası göziçi basıncının iyi kontrolü ve daha da önemlisi enjekte edilen materyalin molekül ağırlığının skleral incelmeyi önlediği düşünülebilir.

Anahtar kelimeler: intravitreal enjeksiyon, sclera, optik koherens tomografi, retina, bevacizumab

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INTRODUCTION

Retinal specialists have been investigating the function of vascular endothelial growth factor (VEGF) and the effects of VEGF inhibitors for nearly a decade in their clinical practices[1,2]. In recent years, intravitreal injection has become the first-line therapy for treating and stabilizing most cases of retinal diseases, such as neovascular age-related macular degeneration, central and branch retinal vein occlusion-related macular edema and diabetic macular edema[3,4]. VEGF appears to be produced by various ocular tissues, including retina, lens and ciliary body. VEGF, released by the pigment epithelium, is a potent promoter of vascular hyperpermeability, and also has an important role in subretinal choroidal neovascularization (CNV) pathogenesis[5,6]. Even though anti-VEGF agents bring innovation the treatment of retinal disorders depending on uncontrolled vascularization, the repeated and long-term injections are commonly needed. At the same time repeated injections increase the risk of ocular and systemic complications. Multiple well-designed studies have demonstrated the local and systemic side-effects of intravitreal anti-VEGF agents, but only one recent retrospective study assessed the scleral thickness changes after intravitreal injections[7].

With the development of ophthalmic imaging techniques, more information can be achieved on the morphologic changes of ocular tissues after disorders and medical interventions. Anterior segment optical coherence tomography (AS-OCT) provides cross-sectional images of the ocular tissues and its image resolution quality is better than ultrasound biomicroscopy [8]. Other advantages of this technique are being rapid and noninvasive [8,9].

The aim of this prospective observational study was to evaluate the changes of scleral thickness after intravitreal injections of bevacizumab in a consecutive series of patients with AS-OCT.

METHODS

The study group consisted of 54 eyes of 54 consecutive phakic patients with retinal pathologies who were indicated for the first time for intravitreal bevacizumab injection at Medipol University School of Medicine, Department of Ophthalmology between February 2015 and November 2015. Only patients followed for at least 12 months were included in this interventional case series design.

Patients with axial length more than 26 mm, connective tissue diseases, previous intraocular surgery (filtrating glaucoma surgery, scleral buckling), ocular trauma, extensive usage of topical steroids for ophthalmologic diseases (uveitis, vernal conjunctivitis) or who were unable to understand the study or communicate were excluded. The study protocol was approved by the Ethics Committee of Medipol University. The tenets of the Declaration of Helsinki were followed and all patients provided informed consent prior to enrollment.

All of the patients were treated with 2.5 mg (0.1 ml) intravitreal injection of bevacizumab (Genentech, San Diego, CA, USA) in superotemporal quadrant of one eye and the fellow eye of each patient formed the control group. To make the standardization between injections, the corneal limbus was marked at 145 and 45 degree with a toric reference marker at while the patient was seated at the surgical table. Injection site was marked 3.5 mm behind from reference point at 145 degree using a surgical caliper. All injections were made with double-plane tunnel technique using a 32 G syringe. Patients were scheduled for monthly follow-up visits after first injection. All patients underwent routine ophthalmic examinations including visual acuity, Goldmann tonometry, slit-lamp biomicroscopy, funduscopy, fluorescein angiography and OCT images at each visit. Re-injection was considered according to fluorescein angiography (vascular leakage) and OCT images (intraretinal or subretinal fluid). Before the first intravitreal injection and after re-injections AS-OCT measurements were performed by experienced technician using a Visante AS-OCT device (Carl Zeiss Meditec AG) with the same technique among both eyes. The Visante is a temporal domain OCT that each image has a maximum transverse and axial optical resolution of 60 μm and 18 μm respectively. The enhanced high-resolution corneal scan mode was used to take scleral cross-sectional images at 135 and 45 degree to capture the four meridians of gaze. During image analysis, the Visante AS-OCT internal software outlines the boundaries of the ocular media and applies corrective refractive indices ($n=1.338$) to obtain best image quality and accuracy. The average of the ten OCT images was taken. The lids were gently retracted by lid retractor, and patients were asked to fixate on a fixation light in four different directions (Inferotemporal, inferonasal, superonasal and superotemporal). Visante AS-OCT on-screen measurement software allows calipers to be super-imposed onto acquired images to extract measurements. The first high reflective tissue signal of the episclera was considered to be the outer limit of the scleral thickness, and the interface

between the sclera (highly reflective) and ciliary body (less reflective) was considered the inner limit [10]. Scleral thickness between 3.5 mm and 4 mm posterior from limbus (L) was measured by two masked observer (S.K.E, M.E) at separate occasions by using the five calipers with a 0.1 mm interval. The averages of ten readings obtained from each observer were calculated and used for subsequent analyses. The axial length (AL) of patients was measured using partial coherence laser interferometry (Zeiss IOL Master; Carl Zeiss AG, Oberkochen, Germany).

Statistical comparisons were done with the paired samples t test and repeated measures ANOVA. Continuous variables were described as mean standard deviation (SD). A p value of 0.05 was considered to be statistically significant.

RESULTS

The study included 54 eyes (32 right, 22 left) from 54 patients (29 females, 25 males). The mean age of patient participants was 68.6 ± 10.1 years (range: 56–80 years). The mean AL was 23.02 ± 1.05 mm in study eyes and 23.24 ± 0.82 mm in fellow eyes ($p=0.367$) whereas the mean IOP was 15.38 ± 1.18 mmHg in study eyes and 15.23 ± 1.73 mmHg in fellow eyes ($p=0.771$). All patients were followed up for 12 months after first IVB injection. The mean injection number was 9.44 ± 0.76 (range: 9–12). The mean of the scleral thickness at each region before and after treatment are displayed in Table 1. Mean baseline scleral thickness of injection site (superotemporal quadrant) and other quadrants (inferonasal, superonasal, inferotemporal) were not statistically different between the injected eyes and fellow non-injected eyes ($p=0.56$, $p=0.63$, $p=0.56$, and $p=0.32$ for superotemporal, inferonasal, superonasal, inferotemporal quadrants, respectively). There was no statistically significant change both at injection site and other quadrants in the study and fellow eye at the end of follow-up period when evaluated with Anova repeated measures (Table 1).

There was also no significant correlation between the changes in scleral thickness and injection numbers ($r=0.43$, $p=0.365$). IVB was well tolerated in all patients. At 1-year follow-up no serious treatment-related ocular complications (iridocyclitis, central retinal vein occlusion, endophthalmitis retinal break or detachment) or non-ocular adverse events (acute elevation of blood pressure, strokes, myocardial infarctions, deaths) were recorded.

Table 1. Clinical characteristics and the mean differences of the initial and twelfth month scleral thickness at each region

	Study Eye	Fellow Eye	p
AL(mm)	23.02±1.05	23.24±0.82	0.367*
IOP(mmHg)	15.38±1.18	15.23±1.73	0.771*
Scleral thickness (µm)preinj			
ST	610.50±22.91	608.17±22.22	0.462†
SN	562.67±27.45	558.02±26.42	0.78†
IN	652.28±18.52	655.82±21.17	0.56†
IT	620.52±26.17	614.37±26.12	0.384†
Scleral thickness (µm)12th month			
ST	604.86±22.39	607.21±19.26	0.319†
SN	559.56±17.25	552.32±20.21	0.56†
IN	650.12±21.32	645.82±23.12	0.63†
IT	618.24±29.14	610.63±26.04	0.325†

AL: axial length, IOP: intraocular pressure, ST: superiotemporal, SN: superionasal, IN: inferionasal, IT: inferotemporal † Anova repeated measures, * paired sample test

DISCUSSION

Since VEGF was described as a key factor in pathogenesis of many retinal disorders, intravitreal injection of anti-VEGF agents such as pegaptanib sodium (Macugen), ranibizumab (Lucentis), aflibercept (Eylea), and bevacizumab (Avastin) have been increasingly used in the treatment of VEGF-mediated retinal diseases. Despite 'unlicensed' use for intraocular treatment, Bevacizumab (full length antibody against VEGF-A) is the most popular agent for treatment of among all the anti-VEGF drugs due to its favorable results and cost effectiveness [12,13]. The current classic treatment of retinal diseases with intravitreal anti-VEGF agents consists of a fixed, monthly dosing regimen [11]. The previous studies suggest that visual outcomes that with anti-VEGF agents after monthly injections for the treatment of neovascularization are better than other available treatment options like laser photocoagulation, photodynamic therapy, and vitreoretinal surgery [14,15]. Although many studies are conducted recently about ocular (damage to the lens, endophthalmitis, retinal detachment, or vascular occlusion) and systemic side effects (arterial hypertension, proteinuria, bleedings, cardiomyopathy, thromboembolic events, and reversible posterior leukoencephalopathy syndrome) of these agents, data about the local side effects of intravitreal anti-VEGF therapy on injection area are still limited in the literature [15,16].

Several studies report conflicting results in anterior scleral thickness because different methods were used [17–19]. OCT has several advantages over other techniques. This technique provides high-resolution images. It is non-contact and it allows rapid image acquisition in the sitting position. It also allows quantitative and dynamic data analysis with high reproducibility and repeatability [20]. Unlike AS-OCT, the supine positioning and contact with the eye cup may alter the natural thickness of the sclera during ultrasound biomicroscopy examination.

Buckhurts et al. [21] conducted an *in vivo* study to measure the anterior scleral thickness in healthy volunteers using the AS-OCT instrument. Scleral cross-section images of 74 individuals were captured in 8 meridians: [superior, inferior, nasal, temporal, superior-temporal, superior-nasal, inferior-temporal and inferior-nasal]. They showed significant differences in anterior scleral thickness between all meridians ($p < 0.001$) except superior vs superior-temporal, inferior-temporal vs inferior-nasal, inferior-temporal vs nasal and inferior-nasal vs nasal. They also indicated that, the mean scleral thickness was maximum on inferior meridian ($806 \pm 60 \mu\text{m}$) and minimum for the superior nasal meridian ($662 \pm 57 \mu\text{m}$).

In 2010 Taban et al. [22] evaluated scleral thickness between the eyes of 12 patients who had unilateral fluocinolone acetonide implants. In this *in vivo* study, they found mean scleral thickness values of 0.99, 0.93, 0.88, 0.86, and 0.92 mm in the inferonasal, inferotemporal, superotemporal, superonasal quadrants, and overall, respectively, in normal eyes using AS-OCT. As a result of this study scleral thickness in the implanted eye was thinner in each quadrant compared to the fellow eyes but the difference was not statistically significant. Furthermore, a trend for decreasing scleral thickness from inferior-nasal followed by inferior-temporal, superior-temporal and superior-nasal was also reported.

Recently Zinkernagel et al. [7] evaluated the effects of anti-VEGF's treatment on scleral thickness among 35 eyes of 35 patients treated with at least 30 intravitreal injections in one eye in the inferotemporal quadrant and 10 or less intravitreal injections in the fellow eye using spectral domain AS-OCT. In this retrospective study, eyes with more than 30 injections the average scleral thickness in the inferotemporal quadrant was $568.4 \mu\text{m}$ and $590.6 \mu\text{m}$ in the fellow eyes with 10 or less injections. The scleral thickness of the eyes with more than 30 injections was significantly thinner than the other eyes. They also compared mean average scleral thickness in the other three quadrants (inferonasal, superotemporal, and superonasal) to detect the effect of over generalized thickness of the sclera and, the difference was not statistically significant. According to their findings, there should be possible additional etiologies in addition to mechanical factors which lead scar formation at injection site. One of them is the direct effect of anti-VEGF antibodies on scleral hydration by reflux through the injection site. In this study ranibizumab and aflibercept were used as an anti-VEGF agent with molecular weight of 48 kDa and 97 kDa respectively. It has been shown that sclera is permeable to molecules with molecular weights up to 150 kDa [23,24].

Therefore, Zinkernagel et al [7] thought that ranibizumab and aflibercept with their molecular weight lower than 150 kDa may be able to diffuse into the sclera through the injection tunnel and may cause dehydration of the sclera by changing the permeability of scleral vessels. In our study bevacizumab was used with molecular weight of 150 kDa and we did not find significant difference in scleral thickness after injection. It might be speculated that due to its higher molecular weight scleral permeability of bevacizumab is limited and it could be the reason of unchanged scleral thickness.

Turgut et al. [25] reported that after removal of the needle following intravitreal injection, vitreous, liquefied vitreous or fluid can reflow through the needle incision into the subconjunctival space. They reported the factors that were significantly associated with reflux of anti-VEGF, including the use of 27 or 30-gauge needles, injection technique, injection quadrant and type of injected anti-VEGF. As a sum of all these factors, reflux could change the dehydration of the sclera by altering the permeability of scleral vessels leading to a localized scleral thinning after repeated injection.

Although a straight needle path, pointing at the center of the eyeball is usually recommended, in order to lower the risk of vitreous reflux, it has been recommended of using sharp small gauge needles (27 gauge needle for Triamcinolone and 30 gauge needle for other drugs) or using a slightly angled scleral path with the injection needle, a short scleral tunnel for injection and pulling the conjunctiva over the in comparing to the previous study could also there should be additional etiologies merit further discussion, there may be a direct effect of anti-VEGF antibodies on scleral hydration. Thus, they believe that the repeated injections of anti-VEGF changes scleral thickness and their study is an initial step towards the understanding of a potential patho-mechanisms leading to scleral changes; especially in the injected quadrant. Intraocular pressure elevation after injections of anti-VEGF agents may be another etiologic factor. The effect of IOP elevation on scleral thickness has been examined in two different experimental studies, one with monkeys and one with human scleral tissue [26,27]. Downs et al. [26] induced high pressure glaucoma in monkeys to investigate the scleral architecture. They reported that significant generalized scleral thinning achieved when IOP levels of 60 mm Hg or higher. Lee et al. [27] also support this hypothesis with their study. According to their study, scleral thickness did show a tendency to decrease as trans-scleral pressure increased to 60 mmHg. However Zinkernagel et al. [7] could not found any significant scleral thinning in the other quadrants.

We attempted to verify previous studies' preliminary results by changing methods and using intravitreal bevacizumab injection. No statistically significant difference was found for scleral thickness before and after nine intravitreal bevacizumab injections. We do not yet know the long term results of our study. However, we think that the well-controlled IOP after intravitreal injections and more important molecular weight of injected material could probably prevent scleral thinning. A further study about the effect of injected material on scleral thickness changes may answer these questions.

Despite limitations such as short follow-up and small number of patients, we think that this study makes important contributions to the advantages of intravitreal usage of bevacizumab.

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