

Pharmacokinetics of bevacizumab after topical and intravitreal administration in human eyes

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Abstract

Background Topical bevacizumab is a potential treatment modality for corneal neovascularization, and several recent studies have demonstrated its efficacy. No previous study of the pharmacokinetics of topical bevacizumab has been performed in human eyes. The purpose of this study is to investigate the pharmacokinetics of topical administration of bevacizumab in human eyes, and also to compare the pharmacokinetics of intravitreal bevacizumab injections with previously reported data.

Methods Twenty-two (22 eyes) were included in this study, and divided into four groups: eight patients received topical bevacizumab and aqueous samples were obtained 1 hour later during cataract extraction surgery (group 1), eight patients received topical bevacizumab and vitreous samples were obtained 1 day later during pars-plana vitrectomy (PPV) (group 2), three patients received intravitreal bevacizumab and vitreous samples were obtained during PPV (group 3). Vitreous samples from three patients who received no bevacizumab served as controls (group 4). All samples underwent enzyme-linked immunosorbent assay to detect bevacizumab.

Results No bevacizumab was detected in the aqueous or vitreous of any topically treated eyes. The mean vitreal half-life for intravitreally injected bevacizumab was 4.9 days in four non-vitrectomized eyes and 0.66 days in one previously vitrectomized eye.

Conclusions Topically administered bevacizumab does not penetrate the cornea into the anterior chamber and vitreous cavity, indicating that topical use for treating corneal neovascularization has minimal risk of intraocular penetration and adverse events related to intraocular vascular endothelial growth factor inhibition. The half-life following intravitreal bevacizumab injection measured in this study is comparable to that of previous reports, and includes the first demonstration of a significantly reduced half-life following intravitreal injection in a previously vitrectomized eye.

Keywords Bevacizumab · Topical · Intravitreal · Pharmacokinetics · Half-life

Introduction

Bevacizumab (Avastin[®], Genentech, San Francisco, CA, USA) is a recombinant humanized monoclonal immunoglobulin antibody specifically directed against human vascular endothelial growth factor (VEGF). It is currently the most widely used anti-VEGF agent in ophthalmology [1, 2]. Bevacizumab is administered intravitreally, most commonly for the treatment of neovascular age-related macular degeneration (AMD), diabetic retinopathy, and retinal vein occlusions [3]. Several studies have demonstrated the efficacy of topical bevacizumab administration for the treatment of corneal neovascularization (NV) in both experimental animal models [4–7] and human patients [8–10].

There are only a few pharmacokinetic studies on topical bevacizumab, and they were performed solely in experimental

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animal models. Nomoto et al. [11] reported minimal aqueous concentration (0.6 ± 0.6 ng/ml) after 1 week of topical administration of 25 mg/ml bevacizumab 6 times daily in rabbit eyes. Yoeruek et al. [12] applied bevacizumab 25 mg/ml drops every minute for 30 minutes to rabbit corneas, and the aqueous penetration after this mega-dose of bevacizumab was minimal, as demonstrated by the fact that the detected amount of bevacizumab was lower by a factor of over 1,000 compared with the initial dose. Dastjerdi et al. [13] reported minimal penetration of topical bevacizumab in normal mice corneas. Others have shown that corneal penetration of bevacizumab was greater in mice with corneal NV and in those with denuded corneal epithelium, and that it can be detected in the aqueous, vitreous, serum, and even in the contralateral eye following subconjunctival injection in several animal models [11, 13, 14]. Kim et al. [14] postulated that intraocular penetration of bevacizumab after subconjunctival injection occurs through the sclera and systemic circulation.

The purpose of this study was to conduct what, to the best of our knowledge, is the first evaluation of the pharmacokinetics of topical bevacizumab in human eyes. We also compared our findings on the pharmacokinetics of intravitreal bevacizumab injection to previously reported data.

Methods

Study subjects

The study protocol followed the tenets of the Declaration of Helsinki, and was approved by the Institutional Review Board of the Tel Aviv Medical Center. All patients agreed to participate after a thorough explanation of the nature of the study, and gave their written informed consent to participate prior to study entry. This prospective study was conducted at the Department of Ophthalmology of the Tel Aviv Medical Center. All patients volunteered to participate in it, and were recruited from among patients scheduled for elective surgery at our department. They were divided into three groups according to bevacizumab treatment protocol and their

subsequent surgery (Table 1): topical bevacizumab and subsequent cataract extraction (group 1), topical bevacizumab and subsequent pars plana vitrectomy (PPV) (group 2), and intravitreal bevacizumab and subsequent PPV (group 3). Control samples were obtained from three additional patients who underwent PPV and had never received any previous treatment with bevacizumab (group 4).

All 22 patients were 18 years or older, and none had corneal neovascularization or epithelial defects prior to PPV or cataract surgery. Only one eye of each patient was included for analysis. The demographic and clinical data that were recorded included age, gender, previous ocular history, previous administration of bevacizumab of any kind, indication for surgery, time between administration of bevacizumab and sampling, and details of the surgery.

Bevacizumab administration

The study participants were divided into four groups. The group 1 patients were treated with topical bevacizumab 25 mg/ml: they received a total of four drops, with 10-minute intervals between drops, 1 hour prior to cataract extraction surgery. The group 2 patients received four drops of topical bevacizumab 25 mg/ml on the day before PPV surgery (once every 6 hours), and an additional four drops 1 hour prior to undergoing surgery at 10-minute intervals. Two patients in group 2 had been treated with intravitreal bevacizumab prior to their inclusion in this study. All group 1 and 2 patients were instructed to perform punctal occlusion for at least 60 seconds after instillation of the bevacizumab drops. The group 3 patients received an intravitreal injection of bevacizumab 1.25 mg/0.05 ml prior to undergoing PPV: the administration of bevacizumab was indicated as part of their treatment, since they all underwent PPV for the removal of vitreous hemorrhage (VH) secondary to proliferative diabetic retinopathy (PDR). The time between bevacizumab injection and vitreous sampling differed between patients in group 3.

The topical and intravitreal bevacizumab administered in this study were prepared under sterile conditions in the pharmacy at our institution. Topical bevacizumab was administered from bottles containing 12 drops of

Table 1 Bevacizumab treatment protocols, surgery type and sampling site for each study group

Group	n	Treatment protocol	Surgery	Sampling site
1	8	Topical — four drops of bevacizumab 25 mg/ml, one drop every 10 minutes 1 hour prior to surgery	Cataract extraction	Aqueous
2	8	Topical — four drops of bevacizumab 25 mg/ml, one drop every 6 hours 1 day before surgery, and another four drops, one every 10 minutes 1 hour prior to surgery	Pars plana vitrectomy	Vitreous
3	3	Intravitreal — injection of bevacizumab 1.25 mg/0.05 ml	Pars plana vitrectomy	Vitreous
4	3	None	Pars plana vitrectomy	Vitreous

• Serum samples were also obtained at the time of surgery in two patients from group 2 and one from group 3.

bevacizumab 25 mg/ml. Syringes containing bevacizumab 1.25 mg/0.05 ml were used for intravitreal administration. Both preparations were refrigerated at 4 °C for no longer than 5 days prior to use.

Surgical procedures and sampling

Cataract extraction surgery was performed by phacoemulsification using the Infinity Vision System (Alcon Laboratories Inc., Fort Worth, TX, USA). All PPVs were 23-gauge, performed using the 23-gauge Constellation Vision System (Alcon Laboratories Inc., Fort Worth, TX, USA). No intraoperative or postoperative complications were encountered. Undiluted aqueous samples were drawn from the group 1 eyes through a paracentesis using a cannula prior to irrigation, while undiluted vitreous samples were drawn through a sclerotomy using the vitrector prior to irrigation in the eyes of the other three groups. Blood samples were also drawn at the time of surgery from three patients in group 2.

Enzyme-linked immunosorbent assay for bevacizumab

Samples obtained from groups 1–4 underwent enzyme-linked immunosorbent assay (ELISA) for bevacizumab. All steps of the ELISA process were performed at room temperature. Nunc 96-well Maxisorp plates (Thermo Scientific, Rockford, IL, USA) were coated with 100 ul/well of hrVEGF (Prospec, East Brunswick, NJ) at 2 ng/ul overnight, followed by blocking at 0.5 % BSA in PBS for 1 hour. Aqueous samples were diluted 1:4, and vitreous and serum samples were diluted 1:2 in 1 % BSA in PBS with 0.05 % Tween 20, and incubated for 2 hours. The wells were then washed with 0.05 % Tween 20 in PBS and incubated for 30 min in goat anti-human IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA, USA) diluted 1:10,000 in 1 % BSA in PBS with 0.05 % Tween 20. The wells were then thoroughly washed before development with 100 ul/well of TMB substrate (Pierce Protein Biology, Rockford, IL, USA) for up to 30 minutes in dark conditions. Absorbance was measured using optical density (OD) values obtained from a microplate reader at 450 nm, with 620 nm as a reference. Bevacizumab concentrations were compared to standard curves generated with 1:2 serial dilutions from 400 ng/ml in 1 % BSA in PBS with 0.05 % Tween 20 with 10 % human serum. This method is very sensitive, and is capable of detecting bevacizumab from a minimal concentration of 6.25 ng/ml. The standard curve was generated from 6.25 ng/ml to 300 ng/ml, using nonspecific human IgG as a negative control in place of the bevacizumab. The detectable range of bevacizumab by this method was therefore between 6.25 ng/ml and 300 ng/ml. For quality control, all drawn samples were divided in two and

analyzed separately. The results of both samples of each patient were then averaged.

Statistical analysis and pharmacokinetic calculations

Data were analyzed using SPSS for windows version 17. Values are presented as mean \pm standard deviation, unless otherwise specified. The bevacizumab concentration was multiplied by estimated volumes of 5.5 ml for vitreous samples and 5,000 ml for serum samples in order to calculate its mass [15, 16]. Half-life ($T_{1/2}$) was calculated according to first-order kinetics.

Results

A total of 22 eyes of 22 patients were included in this study. There were eight males (36.3 %) and 14 females (73.7 %), with a mean age of 69.2 ± 10.2 years (range 53 to 89 years). Their baseline characteristics and indications for surgery are provided in Table 2.

Pharmacokinetics of topical bevacizumab

None of the eight patients in group 1 had received any prior treatment with bevacizumab. Aqueous samples from their eyes were obtained and assayed for bevacizumab. Their OD values were 1.21 ± 0.06 (range 0.038 to 0.246), corresponding with no detectable bevacizumab. The eight patients in group 2 were also treated with topical bevacizumab, but on a longer protocol. Vitreous samples were obtained from their eyes and assayed for bevacizumab. Notably, two of these patients had each received one intravitreal injection of bevacizumab 1.25 mg/0.05 ml prior to their enrollment into this study: patient #11 (Table 2) received the injection 10 weeks prior to surgery, and patient #12 received it 12 weeks prior to surgery.

The OD values in the vitreous samples from the six group 2 patients who had never been treated with bevacizumab were 0.287 ± 0.19 (range 0.059 to 0.636), corresponding with no detectable bevacizumab in any of them. An aqueous sample was available from one of these patients (patient #14), and no bevacizumab was detected in it. Bevacizumab was, however, detected in the vitreous samples from the two patients who were previously treated intravitreally: the vitreal concentration was 92.86 ng/ml in patient #11 and 89.3 ng/ml in patient #12.

No pharmacokinetic calculations could be performed in the aqueous or vitreous samples from patients who were only treated topically, since there was no detectable bevacizumab. The bevacizumab that was detected in the two patients in group 2 was most likely due to the previous intravitreal injections and not to the topical administration in this

Table 2 Baseline characteristics of the study patients

Patient no.	Gender	Age	Group	Surgery	Indication for surgery
1	M	70	1	Phaco	Cataract
2	F	89	1	Phaco	Cataract
3	F	72	1	Phaco	Cataract
4	M	53	1	Phaco	Cataract
5	F	87	1	Phaco	Cataract
6	M	56	1	Phaco	Cataract
7	F	78	1	Phaco	Cataract
8	M	72	1	Phaco	Cataract
9	F	77	2	PPV	ERM
10	M	72	2	PPV	ERM
11	M	69	2	PPV	VH d/t PDR
12	F	75	2	PPV	VH d/t PDR
13	F	63	2	PPV	VH d/t PDR
14	F	66	2	PPV	FTMH
15	M	53	2	PPV	ERM
16	F	73	2	PPV	VH d/t PDR
17	M	59	3	PPV	VH d/t PDR
18	F	73	3	PPV	VH d/t PDR
19	F	64	3	PPV	VH d/t PDR
20	F	82	4	PPV	ERM
21	F	56	4	PPV	ERM
22	F	63	4	PPV	FTMH

M = male; F = female; Phaco = phacoemulsification; PPV = pars plana vitrectomy; ERM = epiretinal membrane; VH d/t PDR = vitreous hemorrhage due to proliferative diabetic retinopathy; FTMH = full-thickness macular hole.

study. The $T_{1/2}$ was 5.7 days for patient #11 and 7.3 days for patient #12.

Pharmacokinetics of intravitreal bevacizumab

In group 3, patients underwent intravitreal injection of bevacizumab 1.25 mg/0.05 prior to PPV for the removal of VH secondary due to PDR. None had been treated by intravitreal bevacizumab prior to inclusion in this study. Vitreous samples were obtained from their eyes and assayed for bevacizumab. The time between intravitreal injection and vitreal sampling varied between these three patients (Table 3), and bevacizumab was detected in the vitreous samples of all of them. In patient #17, PPV had been performed 2 months after intravitreal injection, and a concentration of 19.12 ng/ml of bevacizumab was detected. In patient #18, PPV had been performed 1 month after intravitreal injection, and a concentration of 107.27 ng/ml was detected. The $T_{1/2}$ was 4.1 days for patient #17 and 2.5 days for patient #18.

Patient #19 was previously vitrectomized, and underwent a standard core and peripheral vitrectomy due to a non-clearing

Table 3 Intervals between intravitreal bevacizumab administration and vitreal sampling during pars plana vitrectomy and calculated $T_{1/2}$ values for patients who received intravitreal bevacizumab

Patient	Group	Intervals (days)	Calculated $T_{1/2}$ (days) ^a
11	2	70	5.7
12	2	84	7.3
17	3	56	4.1
18	3	28	2.5
19 ^b	3	7	0.66

^a Half-life ($T_{1/2}$) values for bevacizumab are provided.

^b This patient had previously undergone vitrectomy in the study eye, leading to a significantly reduced $T_{1/2}$ value.

VH 1 year prior to enrollment. The patient was recruited to this study before undergoing repeated PPV for recurrent VH in the same eye, and received an intravitreal injection of bevacizumab 1 week prior to surgery. A concentration of 149.37 ng/ml was detected in the vitreous sample obtained during the study PPV, corresponding with a $T_{1/2}$ of 0.66 days.

No trace of bevacizumab was detected in any of the vitreous control samples from the three patients in group 4.

Systemic pharmacokinetics of topical and intravitreal bevacizumab

Serum samples were available for patient #15 and patient #16 (group 2) who received topical bevacizumab, and no trace of bevacizumab was detected in them. A serum sample was also available for patient #18 (group 3), who received an intravitreal bevacizumab injection 1 month prior to surgery: a sample obtained at the time of surgery contained a serum concentration of 44.85 ng/ml, corresponding with a systemic $T_{1/2}$ of 11.3 days for bevacizumab after intravitreal administration.

Discussion

Bevacizumab was not detected in any of the eyes in which it was administered topically. It was not detected in the aqueous samples of any of the eight patients in group 1, nor in the vitreous samples of the six patients in group 2 who had not been previously treated by intravitreal injection. It was also not detected in serum samples from two patients who were treated solely by topical bevacizumab. These findings are compatible with previous data from animal models, in which corneal penetration was demonstrated as being extremely low, even at higher doses and when following more prolonged treatment protocols [11–14]. An intact corneal epithelium was shown to provide an effective barrier that almost

completely excludes molecules larger than 1 nm [17]. Since bevacizumab is approximately 12 nm long [13], its penetration through healthy corneas is unlikely. Additionally, it has been demonstrated that a monoclonal antibody is unable to penetrate porcine cornea in vitro [18]. No pharmacokinetic calculations could be performed in the current study, since no bevacizumab was detected in eyes that were treated topically. However, our results demonstrate that topical bevacizumab is associated with virtually no penetration in human eyes with intact corneas, corroborating previous findings in experimental animal models.

It should be noted that corneal penetration has been demonstrated to be significantly increased when corneal NV or epithelial defects are present [13]. Several studies suggested that topical bevacizumab may impair corneal epithelial healing and cause stromal thinning after prolonged use [7, 19, 20]. It is also possible that the TWEEN buffer that is present in the commercially available bevacizumab preparation (Avastin®) acts as a detergent that can enhance intraocular penetration [12, 21]. Therefore, it may be assumed that its intraocular penetration will be higher in patients with corneal NV and epithelial defects treated for long periods of time with topical bevacizumab, but several studies have reported very favorable safety profiles for topical administration of bevacizumab in patients with corneal NV [7–9, 22, 23]. An in vitro study demonstrated that the median inhibitory concentration of bevacizumab is 22 ng/ml, and that the minimal concentration that completely blocks all VEGF-induced endothelial cell proliferation, migration, and hyperpermeability is 500 ng/ml [24]. Even if corneal NV and associated epithelial defects increase the risk of bevacizumab penetration, it is probably unlikely to achieve levels that can result in either effective intraocular activity or adverse events. The implications of these findings are encouraging, since corneal NV is an ocular surface disease, and topical administration of bevacizumab will achieve a high local therapeutic level [12, 17], with minimal risk for intraocular or systemic adverse effects related to VEGF inhibition.

Two of our five patients who were treated with intravitreal injection of bevacizumab 1.25 mg/0.05 ml had also been treated with topical bevacizumab according to the group 2 protocol, but the contribution of the latter to the detected level in the vitreous was assumed to be negligible. The time between intravitreal injection and vitreous sampling during PPV and detected concentrations are specified in the Results section and Table 3. Four of these eyes underwent PPV for the first time in this study. Clearance of bevacizumab was calculated to have a $T_{1/2}$ that varied between 2.5 and 7.3 days, with a mean of 4.9 days. These results are compatible with previous pharmacokinetic studies of intravitreal bevacizumab injection in non-vitreotomized human eyes, in which vitreal $T_{1/2}$ varied between 3 and 7.8 days [25–27]. They are also

comparable to previous studies on animal models that reported the $T_{1/2}$ of vitreal bevacizumab between 4.2 and 6.6 days [28–30].

A serum sample was available for one of our patients who underwent intravitreal bevacizumab injection, and a systemic $T_{1/2}$ of 11.3 days was calculated. This value is comparable with a previous animal study that reported a $T_{1/2}$ of 12.9 days [11]. Bevacizumab was not detected in serum samples from two patients who were solely treated topically, demonstrating that topical administration with punctal occlusion is associated with virtually no systemic absorption of bevacizumab.

One eye included in our study had previously been vitreotomized. The detected vitreal concentration of bevacizumab following intravitreal injection in that eye resulted in a calculated $T_{1/2}$ of 0.66 days. This $T_{1/2}$ is significantly shorter than that found in non-vitreotomized eyes, both in our study and in reports by others [25–27]. It is possible that the vitreous serves as a reservoir for injected bevacizumab, and that its absence allows more rapid clearance from the eye. The blood–brain and blood–retinal barriers express neonatal Fc receptor that binds to the Fc portion of antibodies and actively transports them into the systemic circulation [31–33]. In vitreotomized eyes, bevacizumab may more readily be transported out of the eye by this mechanism. This is consistent with previous studies that demonstrated significantly reduced $T_{1/2}$ times after intravitreal injection of triamcinolone acetonide in vitreotomized eyes in both animal models and human eyes [34, 35].

Our search of the literature failed to reveal any publications on the pharmacokinetics of bevacizumab in vitreotomized human eyes. This is a novel finding, which may have significant clinical implications. Bevacizumab is frequently injected into previously vitreotomized eyes in clinical practice, and its increased clearance may mandate more frequent injections. This concept is supported by an earlier study that reported a reduced effect of bevacizumab injections in vitreotomized eyes [36].

Several limitations of this study warrant consideration. First, topical bevacizumab was administered for only 1–2 days, and it is possible that a regimen of more frequent applications for a longer period of time might have resulted in detectable intraocular bevacizumab levels. This is probably unlikely, since the corneal epithelium was intact in all of our studied eyes. Second, since no patients with corneal NV were included, it is not possible to predict the pharmacokinetics of topical bevacizumab in eyes with that pathology. Third, we acknowledge that the number of patients who received intravitreal bevacizumab was small, although there is a precedent to describe as few as two patients in pharmacokinetic studies in human eyes [25–28]. Fourth, we note that the presence of vitreal hemorrhage in eyes that received intravitreal bevacizumab injections may have altered

its pharmacokinetics. However, this has not been established in any study, and since all eyes treated intravitreally had VH, we contend that comparing them is methodologically sound. Finally, fixed volumes were assumed when calculating bevacizumab amounts from vitreal and serum samples. Although estimating individual vitreal and serum volumes from individual patient's axial length and weight might have allowed more accurate calculations, their effect on $T_{1/2}$ calculation would have been very small and clinically insignificant.

In conclusion, our results indicate that topical bevacizumab does not penetrate into the aqueous or vitreous following short-term use. Our intention was to perform pharmacokinetic calculations, but intraocular bevacizumab was not detected following topical administration. These results are consistent with previous studies, and support the growing body of evidence attesting to efficient and safe topical use of bevacizumab. Further studies of the pharmacokinetics in patients with corneal NV are warranted. Additionally, our results on vitreal and systemic $T_{1/2}$ following intravitreal bevacizumab injection corroborate previous studies. Finally, our study demonstrates for the first time that the $T_{1/2}$ of intravitreal bevacizumab in a previously vitrectomized eye is significantly reduced. More comprehensive studies of bevacizumab pharmacokinetics in previously vitrectomized eyes are warranted, and may lead to the formation of different treatment regimens for such patients.

Conflict of interest No author has any proprietary interest in the publication of this report.

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