Intraocular pharmacokinetics of bevacizumab following a single intravitreal injection in humans

Tim U. Krohne, Nicole Eter, Frank G. Holz, and Carsten H. Meyer

Department of Ophthalmology, University of Bonn, Bonn, Germany

Keywords: bevacizumab; Avastin; pharmacokinetics; age-related macular degeneration; diabetic retinopathy; retinal vein occlusion

Corresponding author:
Prof. Dr. med. Carsten H. Meyer
University Eye Hospital
Ernst-Abbe-Str. 2
D-53127 Bonn
Germany
Phone: +49 228 287-15505
E-mail: carsten_h.meyer@ukb.uni-bonn.de
ABSTRACT

PURPOSE: To investigate intraocular concentrations and pharmacokinetics of bevacizumab following a single intravitreal injection in humans.

DESIGN: Prospective, non-comparative, interventional case series.

METHODS: We included 30 non-vitrectomised eyes of 30 patients (age range 43-93 years) diagnosed with clinically significant cataract and concurrent macular oedema secondary to neovascular age-related macular degeneration, diabetic retinopathy or retinal venous occlusion in the same eye. All patients received an intravitreal injection of 1.5mg bevacizumab. Between 1 and 53 days after injection, an aqueous humour sample was obtained during elective cataract surgery. Concentrations of unbound bevacizumab in these samples were quantified by ELISA.

RESULTS: Concentration of bevacizumab in aqueous humour peaked on the first post-injection day with a mean concentration ($c_{max}$) of 33.3µg/ml (range 16.6-42.5µg/ml) and subsequently declined in a monoexponential fashion. Nonlinear regression analysis determined an elimination half-time ($t_{1/2}$) of 9.82 days ($R^2 = 0.81$). No significant differences between diagnosis sub-groups were noted.

CONCLUSIONS: In human non-vitrectomised eyes, the aqueous half-life of 1.5mg intravitreally injected bevacizumab is 9.82 days.
INTRODUCTION

Bevacizumab (Avastin®, Genentech, South San Francisco, CA), a recombinant humanised monoclonal immunoglobulin antibody, is an anti-human vascular endothelial growth factor (VEGF) agent that received approval as an adjunct treatment for colorectal cancer. Intravitreal injections of bevacizumab as off-label use has been shown to be beneficial in eyes with macular oedema secondary to neovascular age-related macular degeneration (AMD), diabetic retinopathy, and central or branch retinal vein occlusion (CRVO/BRVO). However, the pharmacokinetic profile of bevacizumab after intravitreal injection in humans has not yet been clearly determined. With the expanding application of bevacizumab worldwide, it appears prudent to address clearance issues after intravitreal injections in order to optimise dosing regimens of this drug. Beer and co-workers analysed the levels of unbound bevacizumab in two patients 48 hours and 4 weeks, respectively, after a single intravitreal 1.25mg dose. However, there is so far as to the best of our knowledge no published data available on the pharmacokinetics of bevacizumab after intravitreal injection in a human case series. The purpose of this study is to determine the intraocular pharmacokinetics of bevacizumab following a single intravitreal injection in a prospective investigation.

METHODS

PATIENT SELECTION AND SAMPLING: The study received approval by the Institutional Review Board of the University of Bonn. We investigated 30 eyes of 30 patients (mean age 71.1 years, age range 43-93 years, 12 females, 18 males) treated at the Department of Ophthalmology, University of Bonn. Patients agreed to participate in the study by written informed consent.

We included patients who were scheduled for cataract surgery for clinically significant lens opacification and who in addition had previously received intravitreal bevacizumab therapy for macular oedema in the same eye. The macular oedema was secondary to neovascular age-related macular degeneration (AMD) in 6 patients, diabetic retinopathy in 14 patients, and central/branch retinal vein occlusion (CRVO/BRVO) in 10 patients. Only patients who had received a single intravitreal injection of bevacizumab within 60 days before the date of cataract surgery and no additional intravitreal anti-VEGF therapy within 6 months before surgery were included in the study. While prior laser therapy including focal coagulation for treatment of macular oedema was allowed for, patients
with previous intraocular surgery, history of glaucoma, and age below 40 years were excluded.

In all studied patients, bevacizumab injections had been performed in our department following standard procedures. These included the administration of a drop of proparacaine 0.5% and a drop of ofloxacin 0.3% to the eye, followed by injection of 1.5mg bevacizumab into the vitreous cavity 4.0mm posterior to the corneal limbus. Treatment effects were monitored in routine followed-up visits which included optical coherence tomography (OCT) and fluorescence angiography examinations. Based on the examination results, cataract surgery was scheduled when the macular oedema appeared sufficiently controlled, in most cases 2 - 4 weeks after injection. In selected cases, cataract surgery was performed 1 - 2 days following bevacizumab injection in an attempt to prevent surgery-induced reoccurrence or exacerbation of macular oedema in high-risk patients. Overall, the time delay between bevacizumab injection and cataract surgery ranged from 1 to 53 days in the studied patient population.

During cataract surgery, aqueous humour samples were obtained via a corneal paracentesis anterior to the limbus at the beginning of the procedure. The anterior chamber was entered with a 0.5 inch needle on a 1.0ml tuberculin syringe pointing away from the iris. The syringe was filled with approximately 0.15ml fluid from the anterior chamber and immediately stored at -80°C until further analysis.

**BEVACIZUMAB ELISA:** Bevacizumab concentrations in aqueous humour samples were quantified by ELISA. To reduce experimental variability, all samples were measured together in the same assay. Samples were diluted 1:100 in PBS and analysed in triplets. We incubated 100µl of each sample in 96-well plates coated with human IgG-specific goat IgG (BD Biocoat, BD Biosciences, San Jose, CA) for 1 hour at room temperature. Subsequently, wells were incubated for 1 hour with 40ng biotinylated recombinant human VEGF$_{165}$ (Fluorokine Biotinylated Human VEGF, R&D Systems, Minneapolis, MN) in 100µl PBS. This was followed by 100µl peroxidase-conjugated streptavidin (Extravidin-Peroxidase, Sigma, St. Louis, MO) diluted 1:2000 in PBS for 1 hour. Peroxidase activity was determined by incubation with 200µl peroxidase substrate solution (SigmaFast OPD, Sigma). After 10min, absorbance at 450nm was quantified in a microplate reader with subtraction of reference absorbance at 650nm. A standard curve was obtained by serial dilutions of bevacizumab in PBS (Figure 1). The detection range of the ELISA used for analysis comprised 5-500ng/ml. Measurements were performed in triplets and mean results were used for statistical analysis.
STATISTICAL ANALYSIS: A first-order exponential decay equation was fitted to the data by nonlinear regression analysis using SigmaPlot 6.0 software (SPSS, Chicago, IL).

RESULTS

Bevacizumab concentrations were measured in aqueous humour samples obtained from 30 eyes of 30 patients following intravitreal injection of 1.5mg in the same eye (Figure 2). Bevacizumab concentration peaked on post-injection day 1 with a mean concentration \( c_{\text{max}} \) of 33.3µg/ml \( (n = 5, \text{ range } 16.6-42.5\mu g/ml) \). Best fit for the decline of bevacizumab concentration over time was a first-order exponential decay function. Nonlinear regression analysis of the data was performed and reached a determination coefficient \( (R^2) \) of 0.81. According to this analysis, bevacizumab concentration dropped below 10µg/ml at post-injection day 18, below 5µg/ml at day 28, and below 1µg/ml at day 51. Estimated half-time \( (t_{1/2}) \) of bevacizumab elimination from aqueous humour was 9.82 days. Half-time results were consistent in all patient subgroups including neovascular AMD (9.02 days), diabetic retinopathy (10.34 days), and CRVO/BRVO (9.40 days). No significant subgroup-specific differences in overall intraocular bevacizumab levels were noted (Figure 2).

DISCUSSION

To date, only a limited number of reports on the pharmacokinetics of bevacizumab in animal or human eyes has been published. This study provides as to our best knowledge the first investigation on the pharmacokinetics of intraocular bevacizumab in a human case series. In our study encompassing 30 patients, the half-life of bevacizumab in aqueous humour following intravitreal delivery of 1.5mg was 9.82 days. Our findings are in accordance with a yet unpublished study by Csaky and co-workers who report a bevacizumab half-life of 10 days in vitreous samples of 18 patients after injection of 1.25mg (Csaky KG, et al. IOVS 2007;48:ARVO E-Abstract 4936). In rabbits, Bakri and co-workers investigated the pharmacokinetics of 1.25mg intravitreally injected bevacizumab in both vitreous and aqueous and reported elimination half-times of 4.88 days and 4.32 days, respectively.\(^{11}\) A shorter half-life in rabbits compared to humans is in accordance with previous studies on other intravitreally applied drugs like triamcinolone acetonide, reporting a vitreous half-life of 2.89 days in rabbits\(^{12}\) whereas an aqueous half-life of 18.6 days was determined for human eyes.\(^{13}\) This discrepancy may relate to
anatomical differences of rabbit and human eyes including a smaller vitreous volume (approximately 1.5ml vs. 4.5ml).

In this study, patient samples were obtained from the anterior chamber, hence we measured bevacizumab elimination from aqueous humour. While after intravitreal delivery of a drug, aqueous elimination rates are widely considered a good representation for elimination from the vitreous, yet unknown rates of posterior elimination via the choroidal circulation as well as re-entry into the eye from the serum complicate pharmacokinetic modelling for bevacizumab in humans. In the rabbit, two studies investigating intraocular pharmacokinetics of bevacizumab and its derivate drug ranibizumab, respectively, report that elimination from the aqueous humour closely paralleled that from vitreous.\textsuperscript{11, 14} For both drugs, half-live values derived from aqueous humour samples were nearly identical to those obtained from vitreous samples. These results suggest that the aqueous half-life of bevacizumab in humans, as determined in our study, might closely correlate with elimination rate from vitreous.

We excluded eyes with prior vitrectomy from this study because clearance of intravitreally delivered drugs may be markedly accelerated in vitrectomised eyes. Following intravitreal injection of triamcinolone acetonide in rabbits, the half-life was reported to be 2.89 days in non-vitrectomised eyes and 1.57 days in vitrectomised eyes.\textsuperscript{12} Likewise in human eyes, a study analysing aqueous humour samples after intravitreal injection of triamcinolone acetonide determined an elimination half-time of 18.6 days in non-vitrectomised eyes compared to 3.2 days in vitrectomised eyes.\textsuperscript{13} Similar effects would be expected for bevacizumab in vitrectomised eyes and should be considered when clinically applying the drug under these circumstances. Interestingly, recently recent study reported a lack of clinical effect of bevacizumab in vitrectomised human eyes with diabetic macular edema,\textsuperscript{15} possibly related to accelerated clearance of the drug.

On day 1 after injection of 1.5mg bevacizumab, we measured maximal aqueous humour concentrations in our patients with a mean of 33.3µg/ml and a range of 16.6-42.5µg/ml. Despite the use of tunnelled injection technique, varying amounts of drug reflux from the injection site might be responsible for the diverging concentration results. Differences in vitreous volume and resulting drug distribution might be another contributing factor. A similar variability of intraocular concentrations following intravitreal injections has been reported in previous studies.\textsuperscript{13}
In human vitreous, bevacizumab concentrations are reported to reach peak values of 80-170µg/ml (Csaky KG, et al. IOVS 2007;48:ARVO E-Abstract 4936) and 166µg/ml after injection of 1.25mg. These higher concentrations in vitreous compared to our measurements in aqueous humour are to be expected considering the intravitreal delivery of the drug. Indeed, after intravitreal injection of bevacizumab in the rabbit model, maximum concentrations in aqueous humour reach only 9.4% of peak concentrations in vitreous. This ratio is in good accordance with our results in humans.

To reduce experimental variability, we analysed all patient samples together in the same experiment. Until analysis, samples were stored frozen at -80°C for up to 6 months. A recent study demonstrated that a single freeze/thaw cycle does not affect anti-VEGF activity of bevacizumab and that degradation of the frozen drug is minimal over a period of 6 months. For detection of bevacizumab in patient samples, we utilised an ELISA that relies on the binding of bevacizumab to IgG-specific antibodies as well as the binding of labelled VEGF to bevacizumab. This assay principle allows for specific detection of anti-VEGF IgG molecules such as bevacizumab and has been used by previous studies. While this test measures free bevacizumab, it does not detect complexes of bevacizumab with bound VEGF. Assays to detect only the free drug are widely used in pharmacological research and the results are usually considered good representations of total drug concentration. However, as the ratio of VEGF-bound to unbound bevacizumab, the potential effect of the freeze-thaw process on the binding of VEGF to bevacizumab, and the rate of proteolytic bevacizumab degradation in vitreous samples is not known, an underestimation of the total bevacizumab concentration by our results cannot be excluded.

Besides bevacizumab, its derivate drug ranibizumab (Lucentis®, Genentech, South San Francisco, CA) is now widely used as intravitreal anti-VEGF therapy for neovascular AMD. Considering the larger molecular weight of bevacizumab (149kDa), its penetration into the retina and its clearance from the vitreous might be slower compared to the smaller ranibizumab molecule (48kDa). Some authors therefore predict a longer half-life for bevacizumab in the human eye, thus requiring less frequent application. In rabbits, Bakri and co-workers reported a vitreous half-life of 2.88 days following intravitreal injection of 0.5mg ranibizumab, which is indeed shorter than the half-life reported for bevacizumab (4.32 days) in the same animal model. In monkeys, vitreous elimination half-time of ranibizumab determined as 2.6 days by Gaudreault and co-workers after bilateral intravitreal injection of 0.5mg of the drug.
So far, data on vitreous elimination of ranibizumab in humans is only available from the manufacturer's product information.\textsuperscript{19, 21} In a population pharmacokinetic analysis using pooled data from five clinical trials that evaluated intravitreal ranibizumab treatment for neovascular AMD, the elimination rate was determined based on the disappearance of the drug from serum. Vitreous elimination half-time of ranibizumab was estimated to be approximately 9 to 10 days. Comparing these results to our study is difficult due to the different experimental methodology. However, a ranibizumab half-life similar to the half-life of 9.82 days determined for bevacizumab in our study would correspond to our clinical impression that the duration of therapeutic effect does not differ significantly for these two drugs. With the increasing use of intravitreal injections of anti-VEGF substances for various ocular diseases, information on their intraocular elimination kinetics appear prudent and will help to optimise re-injection intervals in order to avoid over- and underdosing.

**ACKNOWLEDGEMENTS:** This study was supported by German Research Council (DFG) Priority Programme “Age-related macular degeneration” (SPP 1088, grant Ho 1926/2-2), and Bonn University BONFOR Programme (Gerok Fellowship, to T.U.K.). The authors thank Claudine Strack, BTA, for expert technical assistance. The study and data accumulation were carried out with approval from the Institutional Review Board (IRB) of the University of Bonn. Informed consent was obtained from the patients. The paper will be presented at the Gonin Club 2008 in St Moritz, Switzerland.

**FINANCIAL DISCLOSURES:** None

**CONTRIBUTIONS OF AUTHORS:** Design and conduct of the study (T.U.K., C.H.M.); selection, treatment, and sampling of patients (N.E., F.G.H., C.H.M.); analysis of samples and statistical evaluation of the data (T.U.K., C.H.M.); interpretation of the data (T.U.K., N.E., F.G.H., C.H.M.); preparation, review, and approval of the manuscript (T.U.K., N.E., F.G.H., C.H.M.)
REFERENCES


FIGURES

FIGURE 1. Bevacizumab ELISA standard curve. Serial dilutions of bevacizumab were measured to calibrate the assay. For sample analysis a detection range of 5 - 500ng/ml (●) was used.

FIGURE 2. Bevacizumab concentrations in aqueous humour following intravitreal delivery of 1.5 mg. Samples were obtained from 30 patients treated for neovascular AMD (○), diabetic macular edema (●), and CRVO/BRVO with secondary macular edema (⊗). Mean values of triplet measurements are plotted both (Left) arithmetically and (Right) semilogarithmically. Regression analysis determined a half-time (t₁/₂) of 9.82 days (R² = 0.81) for elimination of bevacizumab from aqueous humour.