Intraocular pharmacokinetics of ranibizumab following a single intravitreal injection in humans

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ABSTRACT

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

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

METHODS: We included 18 non-vitrectomized eyes of 18 patients (age range, 61 to 85 years) that were diagnosed with both clinically significant cataract and macular edema secondary to either exudative age-related macular degeneration, diabetic maculopathy or retinal vein occlusion. Each eye received a single intravitreal injection of 0.5 mg ranibizumab. An aqueous humor sample was obtained during cataract surgery between 1 and 37 days after injection. Concentrations of unbound ranibizumab in these samples were quantified by enzyme-linked immunosorbent assay.

RESULTS: Ranibizumab concentration in aqueous humor peaked the first day after injection (range, 36.9-66.1 µg/ml) and subsequently declined in a monoexponential fashion. Nonlinear regression analysis determined an initial peak concentration ($c_{\text{max}}$) of 56.1 µg/ml and an elimination half-life ($t_{1/2}$) of 7.19 days with a coefficient of determination ($R^2$) of 0.90.

Correction of ranibizumab concentrations for ocular volume as calculated from axial length measurements did not alter regression analysis results significantly ($t_{1/2}$, 7.15 days; $R^2$, 0.89).

CONCLUSIONS: In human non-vitrectomized eyes, the aqueous half-life of 0.5 mg intravitreally injected ranibizumab is 7.19 days, slightly shorter than the half-life of 9.82 days previously determined for bevacizumab by comparable methods.
INTRODUCTION

Ranibizumab (Lucentis; Genentech, South San Francisco, CA) is a recombinant humanized Fab antibody fragment that blocks human vascular endothelial growth factor (VEGF). It is injected intravitreally as an approved treatment for exudative age-related macular degeneration (AMD), diabetic macular edema (DME), and macular edema secondary to central or branch retinal vein occlusion (CRVO/BRVO). In various other retinal pathologies such as choroidal neovascularization secondary to pathologic myopia and other etiologies and retinopathy of prematurity (ROP) its efficacy is under investigation. Despite this widespread clinical application, the pharmacokinetics of ranibizumab in the human eye are still unknown. Information about its pharmacokinetic properties such as the intraocular elimination half-life is of clinical relevance for optimizing application of the drug and, thus, patient outcome, as well as for comparison with other anti-VEGF agents such as bevacizumab and aflibercept. We have previously reported the pharmacokinetic parameters of bevacizumab in the human eye. Here we determined the pharmacokinetics of ranibizumab in humans using comparable methods.

METHODS

Patient selection and aqueous humor sampling. The study was approved by the Institutional Review Board of the University of Bonn. Patients agreed to participate by written informed consent. We included patients treated at the Department of Ophthalmology, University of Bonn. Inclusion criteria were planned elective cataract surgery for clinically significant lens opacification and a concurrent macular edema secondary to age-related macular degeneration (AMD), diabetic maculopathy, or central or branch retinal vein occlusion (RVO) in the same eye that had been treated with a single intravitreal injection of 0.5 mg ranibizumab within 40 days before surgery. Patients with any additional intravitreal injections within 6 months before surgery or with any previous intraocular surgeries were excluded from the study. During cataract surgery, an aqueous humor sample of
approximately 0.15 ml was obtained via a corneal paracentesis at the beginning of the procedure and immediately stored at -80°C until analysis.

Ranibizumab ELISA. Ranibizumab concentrations in aqueous humor samples were quantified by ELISA, similar as described by us before. To reduce experimental variability, all samples were measured together in the same experiment and each sample was analyzed in triplets. We diluted the samples 1:100 in PBS and incubated 100µl of each diluted 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 VEGF165 (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 30min, 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 ranibizumab in PBS (Figure 1). The ELISA detection range employed for in this study comprised 10-1000ng/ml. Mean results of triplet measurements were used for statistical analysis.

Correction for ocular volume. Axial length of each study eye was measured by optic biometry (IOL Master; Carl Zeiss Meditec, Jena, Germany). Ocular volume was estimated by assuming that the eye approximates a sphere, and thus the following calculation was applied:

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\text{Ocular volume [ml]} = \frac{4}{3} \pi \left( \frac{1}{20} \times \text{axial length [mm]} \right)^3
\]

To correct ranibizumab concentrations for ocular volume, measured concentrations were multiplied by ocular volume to achieve an estimate of the intraocular ranibizumab mass.

Statistical analysis. A mono-exponential decay equation was fitted to the data by nonlinear regression analysis using statistics software (PASW Statistics 18; SPSS, Chicago, IL).
RESULTS

We measured aqueous humor concentration of ranibizumab in 18 eyes of 18 patients (mean age 75.1 years; age range 61-85 years; 10 females, 8 males) who had received an intravitreal injection of 0.5 mg of the drug within a period of 1 to 37 days prior to sampling (Figure 2). Macular edema was secondary to neovascular age-related macular degeneration (AMD) in 9 patients, diabetic maculopathy in 6 patients, and central or branch retinal vein occlusion (RVO) in 3 patients. The highest drug concentration was measured on the first day after injection (n = 2; range 36.9 - 66.1 µg/ml). Best fit for the decline of ranibizumab concentration over time was provided by a mono-exponential decay function. Non-linear regression analysis determined an initial peak concentration ($c_{\text{max}}$) of 56.1 µg/ml, an apparent volume of distribution ($V_d$) of 8.91 ml, and an elimination half-life ($t_{1/2}$) of 7.19 days. The regression analysis reached a coefficient of determination ($R^2$) of 0.90.

To test whether the observed variability of ranibizumab concentrations was due to differences in ocular volume between the study patients, we corrected for ocular volume of each study eye as estimated from measured axial length (Figure 3). However, correction of ranibizumab concentration for ocular volume did not improve the fit of the regression analysis ($R^2$, 0.89) and produced similar half-life results ($t_{1/2}$, 7.15 days).

DISCUSSION

Data on the ocular pharmacokinetics of ranibizumab has so far only been available from animal studies. Hence, previous predictions of pharmacokinetic properties of ranibizumab in humans as well as comparisons with other anti-VEGF agents such as bevacizumab and aflibercept had to rely on estimates of ranibizumab half-life values based on either molecular weight or animal data.9-11 Herein, we quantified ranibizumab concentration in human aqueous humor samples and provide data on the intraocular pharmacokinetics of ranibizumab in humans. We determined an aqueous elimination half-life of ranibizumab in human eyes of 7.19 days which is significantly longer than the respective values in animal models. Similar differences between ocular half-life values in rabbit and monkey animal
models and humans have been observed for bevacizumab\textsuperscript{6, 12, 13} and triamcinolone\textsuperscript{14, 15} and may correspond to the significantly smaller vitreous volume in these animals as compared to humans, underscoring the importance of human studies when analyzing the pharmacokinetics of intravitreally applied drugs.

As there is currently no validated multi-compartmental pharmacokinetic model for intravitreal applied substances, our study employed an one-compartmental model, similar to most previous studies on pharmacokinetics of intravitreal anti-VEGF agents.\textsuperscript{6, 12, 13, 16-20} Our study analyzed aqueous samples obtained during cataract surgery, thus providing data of ranibizumab pharmacokinetics in aqueous humor, not vitreous. In rabbits, Bakri and coworkers reported an aqueous half-life of 2.84 days and a vitreous half-life of 2.88 days.\textsuperscript{16} Another study in rabbits measured aqueous and vitreous half-lives of 3.0 days and 2.9 days, respectively.\textsuperscript{18} In monkeys, Gaudreault and coworkers determined an aqueous half-life of 2.54 days and a vitreous half-life of 2.63 days.\textsuperscript{17} The almost identical aqueous and vitreous values in all these animal models indicate that ranibizumab half-life measurements derived from aqueous samples are a good representation of the respective vitreous values.

As outlined in the product information of ranibizumab, the manufacturer estimated the ocular half-life of the drug from the apparent half-life in serum after intravitreal application, assuming that the systemic half-life of ranibizumab of approximately 2 hours was negligible compared to its ocular half-life.\textsuperscript{21} Using serum samples from patients treated with intravitreal ranibizumab for exudative AMD, the manufacturer estimated the vitreous elimination half-life to be approximately 9 days,\textsuperscript{21} similar to our measured value of 7.19 days.

In a previous study on the VEGF-inhibitory antibody bevacizumab we did not detect any significant differences in intraocular pharmacokinetics between subgroups of patient with AMD, DME, and RVO.\textsuperscript{6} In the present study the number of patients in each diagnosis subgroup was too small to allow for a reliable comparison, and thus we cannot exclude that the pharmacokinetic parameters of ranibizumab reported here may vary in different ocular diseases. In particular in inflammatory conditions an associated impairment of the blood-ocular barriers may affect ocular drug clearance. Similarly, ocular elimination of ranibizumab
would be expected to be significantly accelerated in vitrectomized eyes. As these eyes from our study were excluded this likewise could not be tested for.

Several authors predicted the smaller Fab fragment ranibizumab (48 kDa) to exhibit a shorter ocular half-life compared to the larger full-size antibody bevacizumab (149 kDa). Indeed, Bakri and coworkers compared aqueous half-lives of both drugs in rabbits and found a 0.6-fold shorter half-life of ranibizumab as compared with bevacizumab. In monkeys, Mordenti and coworkers compared vitreous half-lives of Fab-12, an anti-VEGF Fab fragment similar to ranibizumab, and trastuzumab, a full-length antibody like bevacizumab, and likewise detected a 0.6-fold shorter half-life of the Fab fragment. We determined an aqueous half-life of 7.19 days in humans which is 0.7-fold shorter than the half-life of 9.82 days previously reported by us for bevacizumab. Thus our results are in good accordance with those provided by animal studies.

The finding that the about 3-fold larger molecular size of bevacizumab in comparison with ranibizumab results in an only minor increase in ocular half-life may be explained by the activity of the neonatal Fc receptor (FcRn) that is expressed at the blood-brain und blood-retinal barriers. This receptor binds IgG antibodies via their Fc domain on the inner barrier side and transports them in an outward direction into the systemic circulation, possibly as a means of limiting inflammation in the central nervous system. As only the full-size antibody bevacizumab but not the Fab fragment ranibizumab bind to FcRn, clearance of bevacizumab from the eye would be expected to be selectively accelerated by FcRn-mediated transport.

In clinical application, the minimally faster ocular clearance of ranibizumab compared to bevacizumab may be compensated for by a reportedly 5 to 20-fold higher biological activity, resulting in the reported similar therapeutic effect of both drugs when applied in the same monthly intervals and an even slightly lower number of necessary treatments per year for ranibizumab when applied as needed. In addition to this clinical data, pharmacokinetic parameters of ranibizumab as reported in our current study together with our previously
report on bevacizumab pharmacokinetics allow for comparison of both drugs and may help to optimize drug dosing and retreatment intervals.

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REFERENCES


FIGURE LEGENDS

FIGURE 1. Ranibizumab enzyme-linked immunosorbent assay standard curve. The assay employed to determine ranibizumab concentrations in aqueous humor samples was calibrated by serial dilutions of ranibizumab. The detection range of the assay that was used for sample analysis comprised 10 - 1000ng/ml (●).

FIGURE 2. Ranibizumab concentration in aqueous humor over time following intravitreal delivery of 0.5 mg. Samples were obtained from 18 patients treated for exudative age-related macular degeneration (○), diabetic macular edema (●), and central or branch retinal vein occlusion with secondary macular edema (□). Mean values of triplet measurements are
plotted both (Left) arithmetically and (Right) semilogarithmically. Regression analysis determined a half-time ($t_{1/2}$) of 7.19 days for clearance of ranibizumab from aqueous humor. The analysis reached a coefficient of determination ($R^2$) of 0.90.

**FIGURE 3.** Estimated intraocular ranibizumab mass over time following intravitreal delivery of 0.5 mg. To assess whether the pharmacokinetic results obtained from ranibizumab concentration measurements were influenced by variability in ocular volume, we corrected each measured concentration for ocular volume of the respective study eye. For this, ocular volume of each study eye was calculated from measured axial length, and ranibizumab concentration was multiplied by ocular volume to receive an estimate of intraocular ranibizumab mass. Compared to uncorrected concentrations, regression analysis of ocular volume-corrected concentrations yielded similar pharmacokinetic results with a half-life ($t_{1/2}$) of 7.15 days and a coefficient of determination ($R^2$) of 0.89.