

**Impact of silicone oil tamponade on intravitreally injected vancomycin pharmacokinetics
in cynomolgus monkey eyes**

5

Daiki Hira^{1, 2, 4}, Tomoya Kitagawa¹, Taku Imamura³, Masashi Kakinoki³, Satoshi Ueshima¹,
Tomonobu Okano¹, Masahito Ohji³, Mikio Kakumoto¹, Tomohiro Terada^{2,4*}

¹College of Pharmaceutical Sciences, Ritsumeikan University. 1-1-1 Noji-Higashi, Kusatsu,
10 Shiga, 525-8577, Japan, ²Department of Pharmacy, Shiga University of Medical Science
Hospital, Setatsukinowa-cho, Otsu, Shiga, 520-2192, Japan, ³Department of Ophthalmology,
Shiga University of Medical Science, Setatsukinowa-cho, Otsu, Shiga, 520-2192, Japan,
⁴Present address: Department of Clinical Pharmacology and Therapeutics, Kyoto University
Hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan.

15

***Corresponding author:** Tomohiro Terada, Ph.D.

Department of Clinical Pharmacology and Therapeutics, Kyoto University Hospital, 54
Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan.

Tel: +81-75-751-3577, Fax: +81-75-751-4207, E-mail: teradat@kuhp.kyoto-u.ac.jp

Declarations

The animal research protocol was approved by the Animal Experimentation Committee at Shiga University of Medical Science (Approval #2017-8-2).

25 Funding

This work was partly supported by JSPS KAKENHI Grant Number JP20K07211.

Abstract

Intravitreal injections of vancomycin (VCM) and ceftazidime (CAZ) are commonly used to treat infectious endophthalmitis. When patient cases require retinal detachment with silicone oil (SO) tamponade, the antibiotic doses are empirically reduced to 25%. Currently, there is no scientific evidence for these empirical dose reductions. The purpose of the present study is to determine the quantitative impact that SO tamponades have on intraocular VCM pharmacokinetics. Because of high invasiveness of frequent sampling of intraocular VCM concentrations in human, this pharmacokinetic study was performed in cynomolgus monkey's eyes. Population pharmacokinetic modeling and simulation were performed using 75 different intraocular VCM concentrations obtained from 8 male cynomolgus monkeys. A one-compartment model with a first-order diffusion rate was used as a structural pharmacokinetic model. From the covariate analysis, SO tamponade significantly decreased the volume of distribution while pars plana vitrectomy with lensectomy (PPV) significantly increased the clearance and diffusion rate constants. From the Monte Carlo simulation ($n=1,000$), the median time above minimum inhibitory concentration ($T_{>MIC}$, a therapeutic effect index) durations of SO and normal eyes at clinical doses of $1,000\text{ }\mu\text{g}$ were 2.6 and 11.0 days, respectively. Using intravitreal injections of VCM with SO tamponade or PPV may reduce the therapeutic effect.

***Keywords**

silicone oil tamponade; vancomycin; population pharmacokinetics; infectious endophthalmitis;

pars plana vitrectomy; lensectomy

Abbreviations

COPD, chronic obstructive pulmonary disease; VCM, vancomycin; CAZ, ceftazidime; SO, silicone oil; PPV, pars plana vitrectomies; FOCEi, first-order conditional estimation method with interaction; PsN, Perl-speaks-NONMEM; K_D , diffusion rate constant; CL, clearance; V, volume of distribution; OFV, objective function value; OBS, observed concentration; PRED, population predicted value; IPRED, individual predicted value; CWRES, conditional weighted residual error; PK-PD, pharmacokinetics and pharmacodynamics; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; AUC_{0-24} , area under the concentration-time curve form 0-24 h; $T_{>MIC}$, time above MIC; T_{max} , time to maximum VCM concentration; C_{max} , maximum VCM concentration;

1. Introduction

Bacterial endophthalmitis is a major complication of ophthalmic surgeries including intraocular lens implantations and can lead to irreversible vision loss or other serious sequelae. Therefore, serious preventative measures and appropriate treatments are necessary (Barry et al., 2007; Durand, 2013; Luaces-Rodríguez et al., 2018; “Results of the Endophthalmitis Vitrectomy Study: A Randomized Trial of Immediate Vitrectomy and of Intravenous Antibiotics for the Treatment of Postoperative Bacterial Endophthalmitis,” 1995; Todokoro et al., 2018). Vancomycin (VCM), a gram-positive glycopeptide antibiotic and ceftazidime (CAZ),

70 a gram-negative cephem antibiotic, are commonly administered as intravitreal injections to combat *Staphylococcus aureus* and *Klebsiella pneumoniae*, the main pathogens associated with endophthalmitis.(Luaces-Rodríguez et al., 2018; Richette et al., 2015; Tuon et al., 2018) In the case of a retinal tear or detachment from ophthalmic surgery, intraocular silicone oil (SO) tamponade is often used.(Cibis et al., 1962; Meshi et al., 2017; Steel, 2014) Clinically, VCM 75 and CAZ can be administered within the SO injection to patients with retinal detachment, and are often dosed at empirically reduced rate to 25%.(Luaces-Rodríguez et al., 2018) However, little is known about the intraocular dynamics of antibiotics in combination with SO; as such there is little evidence to justify this dose reduction.

Intravitreal pharmacokinetic studies are difficult to conduct in humans as they are very 80 invasive. For this reason, we recently conducted an intravitreal pharmacokinetic study in cynomolgus monkeys. Our study provides evidence showing that the half-lives of VCM and CAZ in the aqueous humor of cynomolgus monkey were significantly shorter in SO-filled eyes than normal eyes.(Imamura et al., 2021) In the present study, a population pharmacokinetic modeling and simulation using our previous data were performed to determine the quantitative 85 impact that empirically reduced drug doses in SO has on intraocular VCM pharmacokinetics.

2. Materials and Methods

2.1. Population pharmacokinetic modeling of intravitreal VCM in cynomolgus monkey

The intraocular VCM concentrations used were determined based on reports by
90 Imamura et al. (Imamura et al., 2021). CAZ was not used in our analysis due to the limited
previous measurements available. The animal research protocol was approved by the Animal
Experimentation Committee at Shiga University of Medical Science (Approval #2017-8-2).

Briefly, eight male cynomolgus monkeys (age 18.3 ± 2.2 y, weight 5.4 ± 0.7 kg) were used.

Four monkeys underwent pars plana vitrectomies (PPV) with lensectomies in their right eyes.

95 A single intravitreal injection of VCM (1 mg/0.1 mL saline) or CAZ (2 mg/0.1 mL saline) was
administered to four normal eyes and four vitrectomized aphakic eyes. VCM and CAZ for
injection were diluted with saline to 1 mg/0.1 mL and 2 mg/0.1 mL, respectively. Aqueous

humor samples (0.1 mL) were obtained just before injection and at 2 and 5 hours on day 0 and
on days 1, 2, 3, 5, 7 and 10 after injection. Three months later, vitreous surgery using a clinically

100 approved silicone oil (1000 centistoke, SILIKONTM 1000 polydimethylsiloxane, Alcon Japan
Ltd, Tokyo, Japan) tamponade was performed on the four vitrectomized aphakic eyes. The
antibiotic administration and sample collection were conducted on the four silicone oil-filled
eyes in the same manner as described above. All samples were stored at -80°C until analysis.

HPLC-UV (Prominence, Shimadzu, Kyoto, Japan) was used to determine the aqueous humor
105 concentrations of VCM. As the deproteinization treatment, 100 μL of acetonitrile containing

6.25 µg/mL of internal standard tinidazole was added to 20 µL of aqueous humor sample. After centrifugation at 12,000 rpm for 10 minutes at 4°C, the supernatant was separated and evaporated to dryness at 40°C. The obtained residue was reconstituted in 200 µL of sodium phosphate buffer (20 mM, pH 2.5). Ten microliters of the reconstituted solution were injected
110 into the HPLC system after filtration. Chromatographic separation was carried out using the Shim-Pack XR-ODS column (75 mm × 3.0 mm) at 40°C. The flow rate was 0.6 mL/min with the following gradient elution. Sodium phosphate buffer (20 mM, pH2.5) and acetonitrile were used as mobile phases A and B, respectively. Then, the percentage of mobile phase B was set as follows: 0–6.5 minutes, 7.2%; 6.51 to 7.50 minutes, 40%; and 7.51 to 16 minutes, 7.2%. The
115 detection wavelength was 257 nm for ceftazidime and 240 nm for vancomycin and tinidazole. The retention times of VCM and IS were 7.0 min and 5.9 min, respectively. In the blank aqueous humor sample, there is no interference peak around these retention times (data not shown). The calibration curve of vancomycin was linear within a range of 0.625–40 mg/L ($R^2 > 0.9995$). The accuracy and precision at lower limit of quantitation (0.625 mg/L) were
120 within 17.6% and 15.0%CV, respectively. The accuracy and precision of three quality control samples (1.875, 25, and 40 mg/L) were within 10.1% and 15.0%CV, respectively. There values were met the acceptance criteria for bioanalytical method validation by US Food and Drug Administration (U.S. Department of Health and Human Services Food and Drug Administration, 2018).

125 A population pharmacokinetic analysis was conducted using a nonlinear mixed-effects
 modeling program (NONMEM 7.4.0, Icon Development Solutions, Ellicott City, MD, USA)
 with a first-order conditional estimation method with interaction (FOCEi) with GFortran.
 Pirana 2.9.9 and Perl-speaks-NONMEM (PsN) were used as modeling workbenches.(Keizer et
 al., 2013) To create a structural pharmacokinetic model for intraocular VCM, a 1-compartment
 130 model with first-order diffusion with no lag time (ADVAN2 TRANS2) was used (**Fig. 1a**). We
 assumed the intravitreally injected VCM was diffused uniformly in the eyeball and aqueous
 humor and was eliminated from the aqueous humor through Schlemm's canal. The mass
 balance equations used for the model are shown below:

$$dX_1/dt = -K_D \times X_1 \quad (\text{Eq. 1})$$

135 $dX_2/dt = K_D \times X_1 - CL/V \times X_2 \quad (\text{Eq. 2})$

K_D is diffusion rate constant from depot compartment to central compartment, CL is clearance
 from the central compartment, V is the volume of distribution within the central compartment
 and X_1 and X_2 are the amounts of the drug in depot and central compartments, respectively.

The inter-individual variability was calculated using an exponential error model as described
 140 below:

$$P_{\text{Individual}} = P_{\text{Population}} \times \exp(\eta_p) \quad (\text{Eq. 3})$$

$P_{\text{Individual}}$ is an individual's estimated parameter value, $P_{\text{Population}}$ is the typical population value
 of the pharmacokinetic parameters such as CL or V , and η_p is an inter-individual random effect

for individuals such that the mean is 0 and the variance is ω_p^2 . A combined proportional and

145 additive error model was examined to calculate the residual error as described below:

$$y_{ij} = F_{ij} \times (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad (\text{Eq. 4})$$

y_{ij} and F_{ij} designate the observed and predicted VCM concentrations in the j th record of i th individual, respectively.

The influence of SO or PPV on pharmacokinetic parameters were calculated using the

150 following equation:

$$P_i = \theta_1 \times \theta_2^{\text{COVi}} \quad (\text{Eq. 5})$$

P_i is a pharmacokinetic parameter for i th individuals and COVi is a dichotomous parameter for either SO or PPV. If the SO tamponade was performed, the dichotomous parameter SO would be equal to 1, otherwise, it would be set to 0. If the vitreous body and crystalline lens were

155 removed by PPV, the dichotomous parameter PPV would be equal to 0, otherwise, it would be set to 1. Covariates were added into the base model using a forward stepwise inclusion approach in order to construct a full model. If a decrease in objective function value (OFV) of more than 3.84 ($p < 0.05$) was observed, it was considered statistically significant. In the second backward step, covariates were removed from the full model. Covariates were kept in the final model if

160 the removal of the covariate resulted in an OFV increase of at least 6.63 ($p < 0.01$).

2.2. Model evaluation

The following diagnostic plots were used to assess the predictive performance of the model: observed concentration (OBS) versus population predicted value (PRED), OBS versus individual predicted value (IPRED), conditional weighted residual error (CWRES) versus PRED and time after the dose. The extent of shrinkage was evaluated with post hoc Bayesian estimate-based diagnostics. The final model was also evaluated with a visual predictive check (VPC) and nonparametric bootstrap analysis. In VPC, a total of 1,000 hypothetical data sets were simulated by NONMEM using the final model to generate the expected concentrations and 90% prediction intervals. The observed data were overlaid on the prediction intervals and compared visually. In the bootstrap analysis conducted using PsN, 1000 bootstrap runs were generated by random resampling of the original data set, and the median and 95% prediction intervals of each parameter were calculated. This analysis included a successful covariance step for calculations. The estimated values of each parameter obtained in the final model were compared to the medians and prediction intervals obtained by the bootstrap method.

2.3. PK-PD simulation after intravitreal VCM injection

A Monte Carlo simulation analysis for the pharmacokinetics and pharmacodynamics (PK-PD) parameter after intravitreal VCM injection was applied to identify the influences of SO or PPV on clinical responses. 1000 different pharmacokinetic parameters for different doses

(250 µg, 1,000 µg, 2,000 µg, 3,000 µg and 4,000 µg) were generated from Monte Carlo simulations that utilized NONMEM® for the final VCM pharmacokinetic model. The PK-PD parameters were calculated based on the generated PK parameter and the minimum inhibitory concentration (MIC) distribution of VCM recorded in clinical isolates of methicillin-resistant

185 *Staphylococcus aureus* (MRSA). According to Japanese national surveillance data,(Takesue et al., 2012) the MIC distribution of VCM against MRSA is as follows: MIC = 0.5 mg/L, 1.9%; MIC = 1.0 mg/L, 88.3%; MIC = 2.0 mg/L, 9.8%. It appears that an area under the concentration-time curve from 0-24 h (AUC_{0-24}) /MIC ≥ 400 is the best predictor of clinical outcomes for intravenous injected VCM.(Kishk et al., 2017; Moise-Broder et al., 2004; Rybak et al., 2009)

190 Here we calculated the $AUC_{0-\infty}$ /MIC in the aqueous humor after a single intravitreal VCM injection. In addition, we also calculated time above MIC ($T_{>MIC}$) because the aqueous humor concentration represents the concentration at the site of action, unlike the serum concentration.(Mouton et al., 2005) $T_{>MIC}$ often represents the cumulative percentage of a 24h period where the drug concentration exceeds MIC,(Mouton et al., 2005) but in the present study,

195 MIC calculations were used instead of percentages for any durations above the MIC since the treatment was a single intravitreal administration rather than a repeated administration.

3. Results

3.1. Population pharmacokinetic modeling of intravitreal VCM in cynomolgus monkey

The pharmacokinetic profiles of intravitreal VCM injections in cynomolgus monkeys are shown in **Fig. 1b**. The time to maximum VCM concentration ($T_{\max}$) in vitrectomized eyes and SO-filled eyes (2 or 5 hours) was shorter than that for normal eyes (24 hours). Conversely, the maximum VCM concentration ($C_{\max}$) in SO-filled eyes was higher than that in vitrectomized eyes and normal eyes. The intravitreal VCM pharmacokinetic profiles were described with a 1-compartment model with first-order diffusion that had no lag time. A covariate analysis (**Table 1**) demonstrated that SO is a significant covariate on V and that PPV is a significant covariate on K_D and CL in the final model (ΔOFV vs base model = -39.2). Pharmacokinetic parameter estimates for the final model are summarized in **Table 2**. The SO tamponade decreased the V of VCM by 32.2%. The PPV increased the K_D and CL of VCM by 76.2% and 38.5%, respectively.

Goodness-of-fit plots (**Fig. 2**) indicated that our final model can adequately predict the VCM concentrations, and that there were no significant miss-specifications in our final model. The nonparametric bootstrap analysis confirmed the homogeneity of variances in our final developed model (**Table 2**). The VPC demonstrated that our simulated concentrations were in reasonable agreement with the observed values (**Fig. 3**).

3.2.PK-PD simulation of intravitreal VCM injection

PK-PD parameters of intravitreal injected VCM were estimated using the individual pharmacokinetic parameters generated by the Monte Carlo simulation analysis (**Fig. 4**). While the PPV decreased the $AUC_{0-\infty}/MIC$, SO tamponade appeared to have no impact on the $AUC_{0-\infty}/MIC$ (**Fig. 4a**). The percentages of $AUC_{0-\infty}/MIC \geq 400$ in the clinical dose (1,000 μg) and empirically reduced (250 μg) groups were 23.4% and 0.0%, respectively. In our simulation, we confirmed that the percentages of $AUC_{0-\infty}/MIC \geq 400$ were 90% or greater anytime the dose was increased above 3,000 μg . The $T_{>MIC}$ was significantly decreased by both PPV and SO tamponade (**Fig. 4b**). The median durations of $T_{>MIC}$ in SO-filled, vitrectomized and normal eyes at a clinical dose (1,000 μg) were 2.6 days, 6.6 days and 11.0 days, respectively. In contrast to $AUC_{0-\infty}/MIC$, $T_{>MIC}$ in SO-filled eyes was not significantly prolonged by increasing the dose. At an increased dose of 4,000 μg in SO-filled eyes, the median $T_{>MIC}$ was 1.2 folds longer (3.2 days) than at a clinical dose (1,000 μg).

4. Discussion

Our study has developed a novel population pharmacokinetic model for the intravitreal injection of VCM in cynomolgus monkeys. Rapid excretion of VCM was observed in macaque eyes with SO tamponade following PPV with lensectomy. The PK-PD simulation from our pharmacokinetic model indicated that $T_{>MIC}$ in SO-filled eyes was significantly shortened, which could possibly result in a decreased clinical response.

As shown in **Fig. 5**, it has been reported that the two major excretion routes of intravitreally injected drugs are the anterior route and the posterior route.(Hutton-Smith et al., 2017; Luaces-Rodríguez et al., 2018; Meredith, 1993) In the anterior route, drugs diffuse from the vitreous humor into the anterior aqueous humor and are excreted into systemic circulation via Schlemm's canal. Therefore, it is likely that hydrophilic or high molecular weight drugs will be excreted via the anterior route. On the other hand, in the posterior route, drugs pass through the retina from the capillaries out to systemic circulation. Therefore, hydrophobic or low molecular weight drugs are likely excreted via the posterior route. It has been reported that VCM is mostly excreted via the anterior route.(Meredith, 1993) Thus, CL and V of intravitreal injected VCM should depend on the aqueous humor flow rate and vitreous volume. In this study, the population estimates of CL and V in normal eyes were 4.07 mL/day (3.23 mL/day - 4.91 mL/day) and 5.08 mL (95%CI: 4.31 mL - 5.85 mL), respectively (**Table 2**). It has been reported that the aqueous humor flow rate and vitreous volume in cynomolgus monkeys were $3.89 \pm$

250 0.40 mL/day (mean $\pm$ SEM)(Wang et al., 2007) and 3.0 - 4.0 mL,(Atsumi et al., 2013; Meredith, 1993) respectively. Though there may be variations depending on the maturity of the monkeys, we consider our obtained population pharmacokinetic parameter estimates to be reasonable. Here, the aqueous humor flow rate and vitreous volume in human were reported 3.60 mL/day and 4.7-5 mL(Missel, 2012), respectively. Since the aqueous humor flow rate is similar but
255 the vitreous volume in human is rather larger than that in cynomolgus monkeys, $T_{>MIC}$ in human may prolonged. However, the reported vitreous volume in human was in the range of the population estimate of V in cynomolgus monkeys, therefore, the possibility of extrapolation to humans requires further investigation.

Covariate analysis showed that V was decreased to 32.2% by SO tamponade (**Table 2**).

260 As described above, the V value likely depends on the vitreous volume (water phase). Since it has been reported that VCM often does not distribute to the SO phase,(Leung and Stout, 2019.) we assume the intraocular water phase should be decreased to approximately 30% with SO tamponade. The reduction of V has been reported to lead to a higher intraocular concentration in SO-filled eyes in other drugs as well as VCM,(Falavarjani et al., 2010; Talwar et al., 2003.)
265 but our study is the first to describe the quantitative impact of SO tamponade based on population pharmacokinetic modeling. We expect that there is a wide variation in the amount of filling in the SO depending on the severity of retinal detachment and the surgeon's preferences, therefore since a wide variability of the SO impact (95%CI: 22.0% - 42.0%) was

observed, it is possible that a more accurate model could be constructed that accounts for the variability in SO injection amounts. In the present study, a 1-compartment model with first-order diffusion with no lag time (**Fig. 1a**) was used to describe the intraocular VCM pharmacokinetics, because VCM does not distribute to the SO phase. If the VCM could distribute to the SO phase, the slope of the elimination phase in SO-filled eyes should be biphasic. Since the slope of the elimination phase in SO-filled eyes was monophasic (**Fig. 1b**), VCM distribution to the SO phase can be ignored. In the PPV eyes, K_D , CL and T_{max} were significantly reduced (**Fig. 1b, Table 2**). Previous studies have reported that a vitrectomy with a lensectomy shortens the half-life of intravitreal molecules.(Christoforidis et al., 2013; Kakinoki et al., 2012; Niwa et al., 2015) It has been suggested that the lens or vitreous body may act as a barrier to the diffusion and excretion of VCM excretion via the anterior route. Therefore, the absence of the lens or vitreous body should also be noted and accounted for.

Since VCM and CAZ inhibit the synthesis of new cell walls, $T_{>MIC}$ may be a good indicator of clinical efficacy.(Zhu and Zhou, 2018) However, VCM in the systemic circulation after intravenous injection has a relatively long half-life and $T_{>MIC}$ tends to be maintained for a long period, so AUC_{24}/MIC is used as an indicator of the clinical outcomes of intravenously injected VCM.(Kishk et al., 2017; Moise-Broder et al., 2004) Therefore, we used simulated $T_{>MIC}$ and $AUC_{0-\infty}/MIC$ values as the PK-PD parameters in the present study. Because $AUC_{0-\infty}$ is calculated by dividing the VCM dose with CL, $AUC_{0-\infty}/MIC$ was decreased by PPV, a

covariate of CL, instead of by the anticipated SO tamponade, a covariate of V. In the PPV-treated eyes, an increased dose of 3,000 μg is required to achieve $\text{AUC}_{0-\infty}/\text{MIC} \geq 400$. Though
290 there was no adverse events assessed by endothelial cell density measurements or electroretinogram recordings with the observed C_{max} range (C_{max} in normal eyes: 151.4 ± 38.5 mg/L, C_{max} in vitrectomized eyes: 205.6 ± 19.7 mg/L, C_{max} in SO-filled eyes: 543.5 ± 187.8 mg/L) in this study (single dose of 1,000 μg), (Imamura et al., 2021) we believe that further dose increases could increase the risk of local side effects in the eyes. (Miyake et al., 2019; Rush et
295 al., 2015; Todorich et al., 2018)

Considering that VCM is rapidly excreted from the eyeball, unlike its excretion from blood, and that the concentration in the aqueous humor equals the concentration at the action site, $T_{>\text{MIC}}$ may be a more useful index than $\text{AUC}_{0-\infty}/\text{MIC}$. As shown in **Fig. 4b**, $T_{>\text{MIC}}$ significantly decreased in SO-filled eyes, therefore, an empirically dose reduction to 250 μg in
300 SO-filled eyes may lead to a less efficacious response and cause drug resistance. Since dose escalation in SO-filled eyes did not prolong $T_{>\text{MIC}}$, repeated intravitreal injections every two or three days are required to achieve the appropriate $T_{>\text{MIC}}$ in SO-filled eyes. However, repeated intravitreal injections are highly invasive unlike intravenous injections, so it may be necessary to develop a controlled release formulation to avoid increasing the number of intravitreal
305 administrations.

One limitation of our study is that it was not conducted in endophthalmitis eyes, though it is reported that the intraocular pharmacokinetics of antibiotics change in endophthalmitis eyes.(Wang et al., 2013) Since the impact of PPV and SO tamponade on the intraocular VCM pharmacokinetics may also change in endophthalmitis eyes, further pharmacokinetic studies should be conducted. Since frequent sampling of aqueous humor in human is difficult due to the invasiveness, we have conducted the pharmacokinetic study in cynomolgus monkey. Therefore, the possibility of extrapolation to humans requires further investigation. It is reported that there are wide interindividual variability in aqueous humor flow rate and vitreous volume in human. (McLaren, 2009; Missel, 2012) These variabilities could increase in real patients compared with that in homogeneous laboratory animals. To extrapolate the present results to real patients in clinical practice, we should take into account various factors such as concomitant drugs that influence the aqueous humor flow rate.

5. Conclusions

Our data suggests that $AUC_{0-\infty}/MIC$ and $T_{>MIC}$ as indexes of therapeutic effects for intravitreally injected VCM for infectious endophthalmitis are reduced by PPV and SO tamponade. We hypothesize that the empirical dose reductions currently used may be inappropriate and may lead to a further reduction of therapeutic effect or an increase in drug

resistance. Our results provide valuable information for the proper use of antimicrobial agents

325 for treating infectious endophthalmitis.

References

- Atsumi, I., Kurata, M., Sakaki, H., 2013. Comparative study on ocular anatomical features among rabbits, beagle dogs and cynomolgus monkeys. *Anim. Eye Res.* 32, 35–41.
- 330 <https://doi.org/10.11254/jscvo.32.35>
- Barry, P., Gettinby, G., Lees, F., Peterson, M., Revie, C., Seal, D., D’Ardis, M., Bradley, B., Grabner, G., Egger, S.F., Ruckhofer, J., Blanckaert, J., Budo, C., Galand, A., Crommen, J., Rakic, J., Xhaufaire, G., Tassignon, M.J., Verbraeken, H., de Donker, R., Schmickler, S., Bellucci, R., Morselli, S., Soldati, S., Vigasio, F., Bertelli, M., Bottoli, A., Cassamali, M., Danieli, F., Khuri, S., Rosa, L., Szaflik, J., Izdebska, J., Szaflik, J.P., 335 Lobo, C., Cunha-Vaz, J., Mira, J., Abreu, A., Aguilar, J., Arteaga, V., Cordovés, L., Díaz-Aleman, V.T., Abreu, P., Alvarez-Marin, J., Gil, M.A., Alió, J., Teus, M., Alvarez, M.T., Román, J.M., Kaynak, S., Saatçý, O., Durak, Ý., Günenç, Ü., Berk, T., Söylev, M., Öner, H., Koçak, N., Ergin, M., Allen, D., Phelan, P., Steel, D., Wood, C., Cunningham, 340 C., Miller, M., Moorthy, R., Zafar, A., Ionides, A., Lake, D., Thompson, G., Jacob, J., Byles, D., Gibbon, C., Kleinschmidt, A., Ling, R., Quinn, A., Simcock, P., Sturrock, G., Kiel, W., Mabey, D., Spalton, D., Kumar, A., Rosen, P., Patel, C.K., Salmon, J., Callens, E., Mathysen, D., Molemans, F., de Rassenfosse, G., Schenkeveld, C., Eckelmann, A., Haselhoff, A., Franczuk, R., Carvalho, L., Catarina, A., Bataille, L., Jiménez, E., de 345 Nicolás, A.M., Kuheylan, H., Bovill, S., Tate, I., Cabral, S., Withers, T., Cox, D.,

Yeates, T., Dash, J., Lindsell, L., Turner, R., Ferrer, C., Koerner, R., Reischl, U., Behr, A., Barrett, S., Gardner, S., Kennedy, S., Ludgate, J., Montan, P., Wilhelmus, K., Barrett, S., Gardner, S., Kennedy, S., Ludgate, J., Montan, P., Wilhelmus, K., 2007. Prophylaxis of postoperative endophthalmitis following cataract surgery: Results of the ESCRS

350 multicenter study and identification of risk factors. *J. Cataract Refract. Surg.* 33, 978–988. <https://doi.org/10.1016/j.jcrs.2007.02.032>

Christoforidis, J.B., Carlton, M.M., Wang, J., Jiang, A., Pratt, C., Abdel-rasoul, M., Hinkle, G.H., Knopp, M. V, 2013. Anatomic and pharmacokinetic properties of intravitreal bevacizumab and ranizumab after vitrectomy and lensectomy. *Retina* 33, 946–952.

355 <https://doi.org/10.1097/IAE.0b013e3182753b12>

Cibis, P.A., Becker, B., Okun, E., Canaan, S., 1962. The use of liquid silicone in retinal detachment surgery. *Arch. Ophthalmol.* 68, 590–599.

<https://doi.org/10.1001/archopht.1962.00960030594005>

Durand, M.L., 2013. Endophthalmitis. *Clin Microbiol Infect* 19, 227–234.

360 <https://doi.org/10.1111/1469-0691.12118>

Falavarjani, G., Modarres, M., Nazari, H., 2010. Therapeutic effect of bevacizumab injected into the silicone oil in eyes with neovascular glaucoma after vitrectomy for advanced diabetic retinopathy. *Eye* 24, 717–719. <https://doi.org/10.1038/eye.2009.94>

- Hutton-Smith, L.A., Gaffney, E.A., Byrne, H.M., Maini, P.K., Gadkar, K., Mazer, N.A.,
 365 2017. Ocular pharmacokinetics of therapeutic antibodies given by intravitreal injection:
 Estimation of retinal permeabilities using a 3-compartment semi-mechanistic model.
 Mol. Pharm. 14, 2690–2696. <https://doi.org/10.1021/acs.molpharmaceut.7b00164>
- Imamura, T., Kakinoki, M., Hira, D., Kitagawa, T., Ueshima, S., Kakumoto, M., Terada, T.,
 Kawamoto, I., Murase, M., Ohji, M., 2021. Pharmacokinetics of intravitreal vancomycin
 370 and ceftazidime in silicone oil-filled Macaque eyes. Transl. Vis. Sci. Technol. 10, 1.
<https://doi.org/10.1167/tvst.10.3.1>
- Kakinoki, M., Sawada, O., Sawada, T., Saishin, Y., Kawamura, H., Ohji, M., 2012. Effect of
 vitrectomy on aqueous VEGF concentration and pharmacokinetics of bevacizumab in
 Macaque monkeys. <https://doi.org/10.1167/iovs.12-10164>
- 375 Keizer, R.J., Karlsson, M.O., Hooker, A., 2013. Modeling and simulation workbench for
 NONMEM: Tutorial on Pirana, PsN, and Xpose. CPT Pharmacometrics Syst.
 Pharmacol. 2, 1–9. <https://doi.org/10.1038/psp.2013.24>
- Kishk, O.A., Lardieri, A.B., Heil, E.L., Morgan, J.A., 2017. Vancomycin AUC/MIC and
 corresponding troughs in a pediatric population. J. Pediatr. Pharmacol. Ther. 22, 41–47.
 380 <https://doi.org/10.5863/1551-6776-22.1.41>
- Leung, E.H., Stout, J.T., 2019. Antibiotics and antifungals in silicone oil. Int J Retina Vitre, 5,
 50. <https://doi.org/10.1186/s40942-019-0199-2>

Luaces-Rodríguez, A., González-Barcia, M., Blanco-Teijeiro, M.J., Gil-Martínez, M.,

Gonzalez, F., Gómez-Ulla, F., Lamas, M.J., Otero-Espinar, F.J., Fernández-Ferreiro, A.,

385 2018. Review of intraocular pharmacokinetics of anti-infectives commonly used in the
treatment of infectious endophthalmitis. *Pharmaceutics* 10, 1–15.

<https://doi.org/10.3390/pharmaceutics10020066>

McLaren J.W., 2009. Measurement of aqueous humor flow. *Exp. Eye. Res.* 88, 641–647.

Meredith, T.A., 1993. Antimicrobial pharmacokinetics in endophthalmitis treatment: Studies

390 of ceftazidime. *Trans. Am. Ophthalmol. Soc.* 91, 653–700.

Meshi, A., Friehmann, A., Sella, S., Gepstein, R., Armarnik, S., Assia, E.I., Rubowitz, A.,

2017. Intravitreal administration of antiviral agents in silicone oil–filled Human eyes.

Ophthalmol. Retin. 1, 288–293. <https://doi.org/10.1016/j.oret.2016.12.006>

Missel, P.J., 2012. Simulating intravitreal injections in anatomically accurate models for

395 rabbit, monkey, and human eyes. *Pharm. Res.* 29, 3251–3272.

<https://doi.org/10.1007/s11095-012-0721-9>

Miyake, H., Miyazaki, D., Shimizu, Y., Sasaki, S. ichi, Baba, T., Inoue, Y., Matsuura, K.,

2019. Toxicities of and inflammatory responses to moxifloxacin, cefuroxime, and

vancomycin on retinal vascular cells. *Sci. Rep.* 9. <https://doi.org/10.1038/s41598-019->

400 46236-2

Moise-Broder, P.A., Forrest, A., Birmingham, M.C., Schentag, J.J., 2004. Pharmacodynamics of vancomycin and other antimicrobials in patients with *Staphylococcus aureus* lower respiratory tract infections. *Clin. Pharmacokinet.* 43, 925–942.
<https://doi.org/10.2165/00003088-200443130-00005>

405 Mouton, J.W., Dudley, M.N., Cars, O., Derendorf, H., Drusano, G.L., 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: An update. *J. Antimicrob. Chemother.* 55, 601–607. <https://doi.org/10.1093/jac/dki079>

Niwa, Y., Kakinoki, M., Sawada, T., Wang, X., Ohji, M., 2015. Physiology and pharmacology ranibizumab and aflibercept: Intraocular pharmacokinetics and their effects on aqueous VEGF level in vitrectomized and nonvitrectomized Macaque eyes.
410 <https://doi.org/10.1167/iovs.15-17279>

Results of the endophthalmitis vitrectomy study: A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis, 1995. *Arch. Ophthalmol.* 113, 1479–1496.

415 <https://doi.org/10.1001/archophth.1995.01100120009001>

Richette, P., Flipo, R.N., Patrikos, D.K., 2015. Characteristics and management of gout patients in Europe: data from a large cohort of patients. *Eur Rev Med Pharmacol Sci* 19, 630–639.

- Rush, S.W., Vu, D., Rush, R.B., 2015. The safety and efficacy of routine administration of
 420 intracameral vancomycin during cataract surgery. *J. Ophthalmol.* 2015.
<https://doi.org/10.1155/2015/813697>
- Rybak, M., Lomaestro, B., Rotschafer, J.C., Moellering, R., Craig, W., Billeter, M.,
 Dalovisio, J.R., Levine, D.P., Reilly, C., 2009. Therapeutic monitoring of vancomycin in
 adult patients: A consensus review of the American Society of Health-System
 425 Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious
 Diseases Pharmacists. *Am. J. Heal. Pharm.* 66, 82–98.
<https://doi.org/10.2146/ajhp080434>
- Steel, D., 2014. Retinal detachment. *BMJ Clin. Evid.* 2014: 0710.
- Takesue, Y., Watanabe, A., Hanaki, H., Kusachi, S., Matsumoto, T., Iwamoto, A., Totsuka,
 430 K., Sunakawa, K., Yagisawa, M., Sato, J., Oguri, T., Nakanishi, K., Sumiyama, Y.,
 Kitagawa, Y., Wakabayashi, G., Koyama, I., Yanaga, K., Konishi, T., Fukushima, R.,
 Seki, S., Imai, S., Shintani, T., Tsukada, H., Tsukada, K., Omura, K., Mikamo, H.,
 Takeyama, H., Kusunoki, M., Kubo, S., Shimizu, J., Hirai, T., Ohge, H., Kadowaki, A.,
 Okamoto, K., Yanagihara, K., 2012. Nationwide surveillance of antimicrobial
 435 susceptibility patterns of pathogens isolated from surgical site infections (SSI) in Japan. *J*
Infect Chemother. 18, 816–826. <https://doi.org/10.1007/s10156-012-0509-1>

Talwar, D., Kulkarni, A., Azad, R., Gupta, S.K., Velpandian, T., Sharma, Y., Biswas, N.R.,
2003. Intraocular ciprofloxacin levels after oral administration in silicone oil-filled eyes.

Invest Ophthalmol Vis Sci. 44, 505–509. <https://doi.org/10.1167/iovs.02-0499>

440 Todokoro, D., Mochizuki, K., Nishida, T., Eguchi, H., Miyamoto, T., Hattori, T., Suzuki, T.,

Inoue, T., Nejima, R., Hoshi, S., Akiyama, H., 2018. Isolates and antibiotic
susceptibilities of endogenous bacterial endophthalmitis: A retrospective multicenter
study in Japan. J. Infect. Chemother. 24, 458–462.

<https://doi.org/10.1016/j.jiac.2018.01.019>

445 Todorich, B., Faia, L.J., Thanos, A., Amin, M., Folberg, R., Wolfe, J.D., Todorich, K.M.,

Raphtis, E., Ruby, A.J., Williams, G.A., Hassan, T.S., 2018. Vancomycin-associated
hemorrhagic occlusive retinal vasculitis: A clinical-pathophysiological analysis. Am. J.
Ophthalmol. 188, 131–140. <https://doi.org/10.1016/j.ajo.2018.01.030>

Tuon, F.F., Rocha, J.L., Formigoni-Pinto, M.R., 2018. Pharmacological aspects and spectrum

450 of action of ceftazidime–avibactam: a systematic review. Infection.

<https://doi.org/10.1007/s15010-017-1096-y>

U.S. Department of Health and Human Services Food and Drug Administration, 2018.

Guideline for industry bioanalytical method validation. <https://doi.org/10.5958/2231-5675.2015.00035.6>

- 455 Wang, B., Nevins, J.E., Nadler, Z., Wollstein, G., Ishikawa, H., Bilonick, R.A., Kagemann, L., Sigal, I.A., Grulkowski, I., Liu, J.J., Kraus, M., Lu, C.D., Horneegger, J., Fujimoto, J.G., Schuman, J.S., 2013. In vivo lamina cribrosa micro-architecture in healthy and glaucomatous eyes as assessed by optical coherence tomography. *Invest. Ophthalmol. Vis. Sci.* 54, 8270–8274. <https://doi.org/10.1167/iovs.13-13109>
- 460 Wang, R.F., Gagliuso, D.J., Mittag, T.W., Podos, S.M., 2007. Effect of 15-keto latanoprost on intraocular pressure and aqueous humor dynamics in monkey eyes. *Investig. Ophthalmol. Vis. Sci.* 48, 4143–4147. <https://doi.org/10.1167/iovs.07-0035>
- Zhu, L.L., Zhou, Q., 2018. Optimal infusion rate in antimicrobial therapy: Explosion of evidence in the last five years. *Infect. Drug Resist.* <https://doi.org/10.2147/IDR.S167616>

465

Figure legends

Fig. 1. (a) Schematic diagrams of the intraocular VCM pharmacokinetic model and (b)

470 **aqueous humor concentration-time profiles of VCM after intravitreal injection in**
cynomolgus monkeys. K_D is diffusion rate constant. Blue rhombuses, red circles and black
squares indicate vitrectomized eyes, silicone oil-filled eyes and normal eyes, respectively ($n =$
4 in each group).

475 **Fig. 2. Goodness-of-fit plots for the final models of VCM.** The relationship between the
observations (OBS) and (a) population prediction (PRED), and (b) individual prediction
(IPRED), conditional weighted residual (CWRES) and (c) PRED and (d) the time after the dose.
Open circles indicate the observed values. Each line shows a line of identity.

480 **Fig. 3. Visual predictive check of VCM concentrations with data obtained from the final**
model in (a) vitrectomized eyes, (b) silicone oil-filled eyes and (c) normal eyes. Open circles
indicate the observed values. The solid, and dashed lines indicate the simulation-based median,
5th and 95th percentiles, respectively.

485 **Fig. 4. Simulated (a) $AUC_{0-\infty}/MIC$ and (b) $T_{>MIC}$ after intravitreal VCM injection in (A)**
vitrectomized eyes, (B) silicone oil-filled eyes and (C) normal eyes. Dashed line indicates
the $AUC/MIC = 400$.

Fig. 5. Schematic diagrams of excretion of intravitreal injected drugs (a) without or (b)
490 **with concomitant use of silicone oil.** Black and white arrows indicate the anterior route for
hydrophilic agents, and the posterior route for hydrophobic agents, respectively.

Table 1 Summary of covariate selection step.

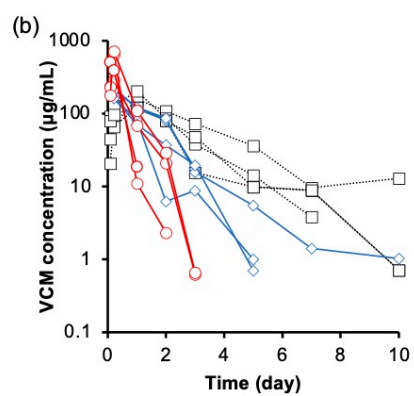
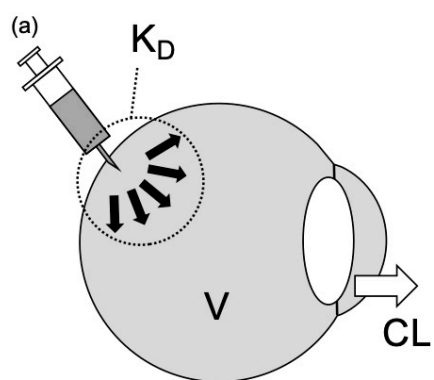
	No.	Equation	OFV	Δ OFV vs base model
Base model	#1	No covariate	559.354	-
	#2	#1 + SO on V	542.256	-17.1
Forward addition	#3	#1 + PPV on V	548.654	-10.7
	#4	#1 + PPV on CL	555.389	-4.0
	#5	#1 + PPV on K _D	548.118	-11.2
Full model	#6	#1 - (#2 + #3 + #4 + #5)	518.291	-41.1
				Δ OFV vs full model
	#7	#6 -SO on V	535.555	17.3
	#8	#6 -PPV on V	520.106	1.8
	#9	#6 -PPV on CL	523.229	4.9
Backward elimination	#10	#6 -PPV on K _D	528.44	10.1
				Δ OFV vs. #8 model
	#11	#8 -SO on V	540.447	20.3
	#12	#8 -PPV on CL	527.635	7.5
	#13	#8 -PPV on K _D	536.798	16.7
				Δ OFV vs base model
Final model	#14	SO on V + PPV on CL and K _D	520.106	-39.2

495 K_D: diffusion rate constant, OFV: objective function value, SO: silicone oil, PPV: pars plana vitrectomy

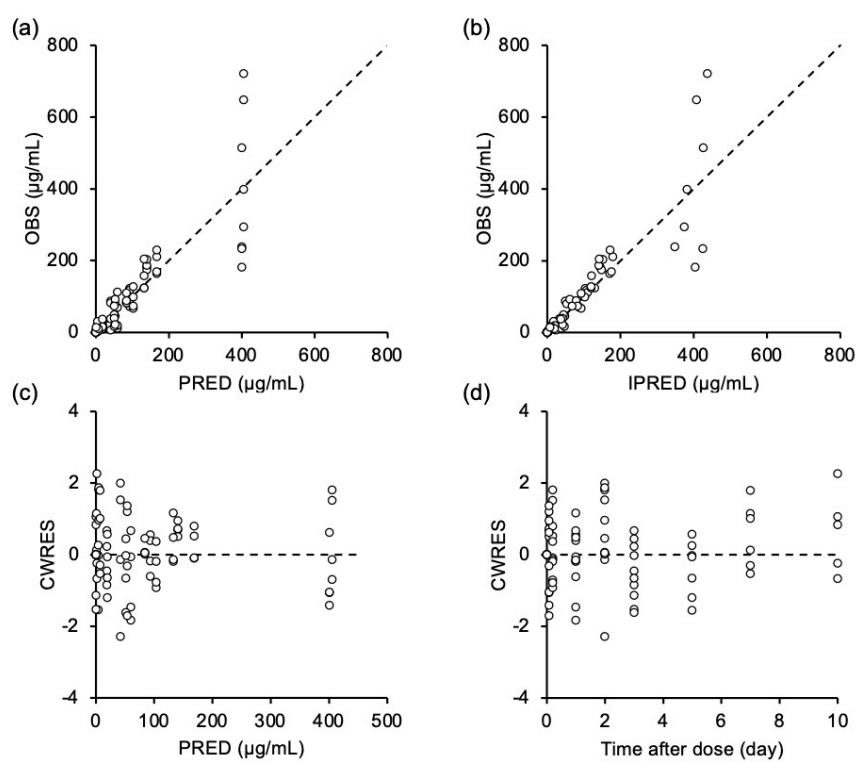
Table 2 Population pharmacokinetic parameter estimates for VCM after intravitreal injection in cynomolgus monkeys.

Parameters	Final model		Bootstrap (n = 1000)	
	Mean	95% CI	Median	95% CI
$V \text{ (mL)} = \theta_1 \times \theta_4^{SO} \times \exp(\eta_V)$				
$\theta_1 \text{ (mL)}$	5.08	4.31–5.85	5.02	4.34–5.83
θ_4	0.322	0.222–0.422	0.322	0.214–0.490
$CL \text{ (mL/day)} = \theta_2 \times \theta_5^{PPV} \times \exp(\eta_{CL})$				
$\theta_2 \text{ (mL/day)}$	4.07	3.23–4.91	4.02	3.15–4.89
θ_5	0.615	0.457–0.773	0.604	0.469–0.776
$K_D \text{ (/day)} = \theta_3 \times \theta_6^{PPV}$				
$\theta_3 \text{ (/day)}$	16.3	8.7–23.9	16.2	10.0–26.7
θ_6	0.239	0.111–0.367	0.230	0.122–0.377
Inter- and intra- individual variabilities				
	Mean (RSE %) [shrinkage %]		Median	95% CI
$\eta_V \text{ (%)}$	16.6 (106.9) [28.4]		17.3	4.8–32.9
$\eta_{CL} \text{ (%)}$	21.2 (42.4) [7.2]		17.5	7.5–26.8
$\varepsilon_1 \text{ (%)}$	38.5 (21.8) [8.6]		36.2	24.2–44.4
$\varepsilon_2 \text{ (%)}$	83.2 (57.1) [8.6]		89.9	56.1–414.4

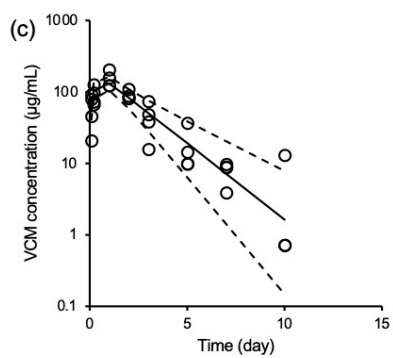
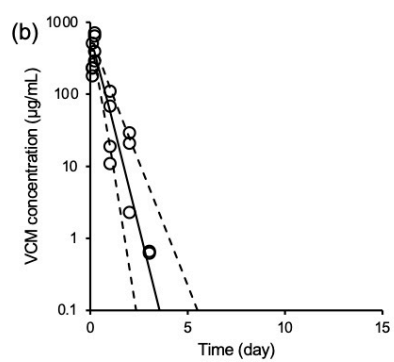
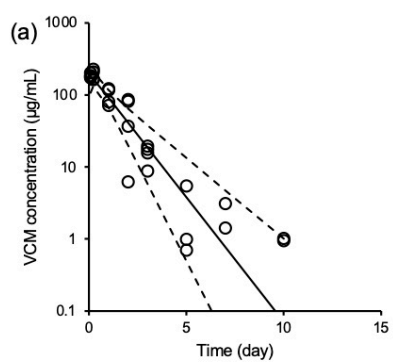
500 If silicone oil (SO) was used, the dichotomous parameter SO was equal to 1, otherwise it would
be set to 0. If the vitreous body and crystalline lens were removed by pars plana vitrectomy
(PPV), the dichotomous parameter PPV was equal to 0, otherwise it would be set to 1. The η_V
and η_{CL} indicate the inter-individual variabilities of V and CL, respectively. The ε_1 and ε_2
indicate the intra-individual variabilities for proportional and additive errors, respectively.
505 Success rate of 1000 bootstrap was 99.3%.



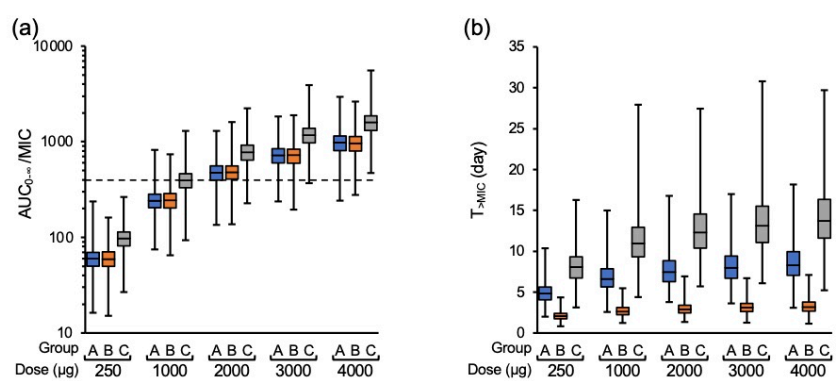
Hira et al., Fig. 1



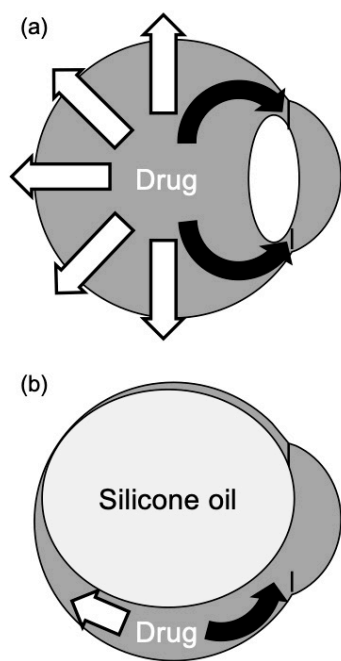
Hira et al., Fig. 2



Hira et al., Fig. 3



Hira et al., Fig. 4



Hira et al., Fig. 5