Determining a No-Observable Effect Level for Endotoxin Following a Single Intravitreal Administration to Dutch Belted Rabbits

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Submitted: December 21, 2016
Accepted: February 11, 2017
Citation: Bantsev V, Miller PE, Bentley E, et al. Determination of a no-observable effect level for endotoxin following a single intravitreal administration to Dutch belted rabbits. Invest Ophthalmol Vis Sci. 2017;58:1545–1552. DOI:10.1167/iovs.16-21356

Purpose. The purpose of this study was to characterize the inflammatory response and determine a no-observable effect level (NOEL) in rabbit eyes after endotoxin intravitreal (ITV) injection.

Methods. Fifty-three naïve male Dutch Belted rabbits were treated with a single 50-μL ITV injection ranging from 0.01 to 0.75 endotoxin units/eye (EU/eye) and monitored for up to 42 days post treatment. Ophthalmic examination included slit-lamp biomicroscopy and indirect ophthalmoscopy. Laser flare photometry was performed in a subset of animals. On days 2, 8, 16, and 43, a subset of animals was necropsied and eyes processed for histopathological evaluation.

Results. Intravitreal injection of endotoxin at ≥0.05 EU/eye resulted in a dose-related anterior segment inflammation response. No aqueous flare or cell response was noted in the 0.01 EU/eye dose group. A more delayed posterior segment response characterized by vitreal cell response was observed beginning on day 5, peaking on day 9, and decreasing starting on day 16 that persisted at trace to a level of 1+ on day 43. Microscopy findings of infiltrates of minimal mixed inflammatory cells in the vitreous and subconjunctiva and proteinaceous fluid in the anterior chamber and/or vitreous were observed in eyes given ≥0.1 EU/eye.

Conclusions. We defined the NOEL for ITV endotoxin to be 0.01 EU/eye, suggesting that the vitreal cavity is more sensitive to the effects of endotoxin than the anterior segment and aqueous chamber. These data highlight the importance of assessing endotoxin level in intravitreal formulations, as levels as low as 0.05 EU/eye may confound the safety evaluations of intravitreal therapeutics in rabbits.

Keywords: drug development, endotoxin, intravitreal injection, ocular toxicity

Intravitreal injections (ITV) were first introduced in 1911, initially as ITV of air for the purpose of repairing retinal detachments.1 Since that time, ITV have been used for treatment of a variety of conditions, including endophthalmitis, intraocular lymphoma, cytomegalovirus retinitis, submacular hemorrhage, and vitreous hemorrhage. In the last 15 years, ITV administration of therapeutic drugs has become increasingly common, driven mainly by the successful development of steroid and anti-VEGF therapies for treatment of neovascular age-related macular degeneration. Intravitreal injections have now become a cornerstone of retinal care and are commonly performed in ophthalmology.2 The primary benefit of ITV is direct and local delivery of a therapeutic agent to the vitreous and retina while minimizing systemic exposure.

One of the challenges presented by the increased use of this route of drug administration is understanding the key product quality attributes for ITV formulations. Perhaps above all other potential contaminations, endotoxin is a major concern for these products. Bacterial endotoxin is a lipopolysaccharide released from the wall of gram-negative bacteria. Endotoxin is highly heat resistant and is difficult to eliminate from sterile surgical instruments or formulations. The eye has been shown to be very sensitive to the effects of intracameral endotoxin and endotoxin-contaminated ophthalmic viscosurgical devices (OVDs).3-5 The inflammatory effects of endotoxin are well described, and it is often used to produce experimental animal models of uveitis following ITV in mice,6 rabbits,7-11 pigs,12 and monkeys.13 Clinically, cases of acute postoperative sterile uveitis or toxic anterior segment syndrome have been linked to endotoxin contamination of ophthalmic devices.14-16 Additionally, therapeutic biologics for intravitreal administration may be at increased risk for contamination with endotoxin or other biologic impurities, particularly if they are produced in prokaryotic expression systems.

Despite the concerns regarding endotoxin contamination of ophthalmology products and the emerging importance of ITV injections as a route of drug administration, little is known about the sensitivity and time course of the response to
endotoxin following ITV administration. Characterization of this response would allow for a more informed risk assessment of novel ITV formulations. Importantly, such data would also allow the discrimination of drug-related effects from endotoxin-related effects in nonclinical safety studies for biologic therapeutics, where nonspecific inflammation response to a humanized protein is often observed. The purpose of this 43-day study was to define the response to intravitreal purified reference endotoxin levels of 0.01 to 0.75 endotoxin unit/eye (EU/eye) in Dutch Belted rabbits, including the definition of a no-obsorable effect level (NOEL) and the time course of the response in both the anterior and posterior segments of the eye. As mentioned above, adverse ocular effects following intracameral endotoxin and endotoxin-contaminated OVDs in rabbits have been described previously,3-5 and generally was used as the basis for dose range selection in the current study.

**MATERIALS AND METHODS**

**Test Species**

Fifty-three naïve male Dutch Belted rabbits (Covance Research Products, Inc., Denver, PA, USA) were assigned to the study. The local animal care committees (Covance Laboratories, Inc. or Genentech, Inc.; San Francisco, CA, USA), in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, approved all animal protocols. Animals were at least 4 months of age and ranged in weight from 1.4 to 2.8 kg at study initiation. Each animal was housed individually in a stainless steel cage in an environmentally controlled room. Food consisted of 120g/day commercial food (Certified Rabbit Diet 5322; PMI Nutrition International Certified LabDiet, St. Louis, MO, USA) and water ad libitum, along with supplemental dietary enrichment.

**Test Materials**

Purified reference endotoxin (i.e., lipopolysaccharides from Escherichia coli) was obtained (reference standard lot HOK354; United States Pharmacopeia, Rockville, MD, USA) and reconstituted to a stock concentration of 2000 EU/mL using limulus amoebocyte lysate assay reagent-grade water. Endotoxin stock was diluted to appropriate concentrations for dosages, using phosphate buffered saline (Sigma-Aldrich Corp., St. Louis, MO, USA). All dose preparations were apportioned on the day of use and stored in a refrigerator set to maintain temperatures from 2°C to 8°C, until released for dose administration. Dose syringes were filled under a laminar flow hood. Within 4 hours prior to dose administration, vehicle control and test article solutions were drawn through a 0.3-mL insulin syringe with a 29-gauge needle attached for ITV injection. Dose preparation concentrations were analyzed using a kinetic limulus amoebocyte lysate assay with a four-point standard curve (0.005 to 5.0 EU/mL). Reagent kits were obtained from Charles River Laboratories (Boston, MA, USA), and data were analyzed using WinKQCL software (Walkersville, MD, USA).

**Dose Administration**

Intravitreal injections were performed by a board-certified veterinary ophthalmologist. Prior to the ITV injections, animals were anesthetized with intramuscular injections of ketamine (20 to 30 mg/kg), dexmedetomidine (0.0315 mg/kg), and glycopyrrolate (0.01 mg/kg). A topical anesthetic (e.g., 0.5% proparacaine) was instilled in each eye before the dose administration. A wire speculum was used to retract the eyelids. To minimize external ocular irritation, eye preparation was limited to a dose site-specific cleaning with dilute 1% povidone iodine solution (prepared with sterile saline and 5% povidone iodine) and rinsed with sterile saline prior to the dose administration.

Doses were administered by ITV injection, 50 μL per eye (Table). A 0.3-mL insulin syringe with a 29-gauge needle attached was used for each dose administration. Injections were administered in the superior temporal region of each eye, which corresponded in the right eye to approximately the 11 o'clock position and in the left eye to approximately the 1 o'clock position. A topical antibiotic (Tobrex, Alcon Laboratories, Inc., Fort Worth, TX, USA) was instilled in each eye following dosing.

**Medication Regimen**

Upon recovery from anesthesia, each rabbit received a sustained-release opioid (buprenorphine, 0.2 mg/kg) by subcutaneous injection, as well as an oral formulation of tramadol (an opioid) administered at a dose of 4 mg/kg. A second dose of tramadol at the same dose level was administered at least 6 hours after the first tramadol administration. Additional buprenorphine (nonsustained release) and bland ophthalmic ointment were administered as needed by the veterinary staff.

**Ophthalmic Procedures**

Ophthalmic examinations, IOP and laser flare photometry (LFP) were performed pre dose and at 4 and 8 hours post dose, and on days 2, 3, 4, 5, 8, 9, 12, 16, 22, 29, 36, and 43 (Table).

Ophthalmic examinations were performed by a board-certified veterinary ophthalmologist. The anterior segments of both eyes of each animal were examined first, using a handheld slit-lamp biomicroscopy (on undilated eyes), followed by table-mounted slit-lamp examination (of dilated eyes) and indirect ophthalmoscopy. Slit-lamp evaluation included examination of the conjunctiva, cornea, anterior chamber, iris, pupillary reflex, lens, and anterior vitreous. The eyes were scored based on a modified Hackett-McDonald scoring system. The Standardization of Uveitis Nomenclature (SUN) Working Group grading scheme17 was used to score anterior chamber flare and cell, using a table-mounted slit lamp with the field for evaluation of approximately 1-mm diameter spot followed by a second hand-help slit-lamp biomicroscopy examination. Pupils were pharmacologically dilated using tropicamide prior to indirect ophthalmoscopy. Interocular pressure values were measured in triplicate, using a rebound tonometer (TonoVet; Icare, Vantaa, Finland). Only values with no or insignificant deviation (steady letter) were used.

Following the ophthalmic examinations and IOP measurements for each animal, aqueous flare was measured using a laser flare meter (model FM-600; Kowa Optimed, Inc., Torrance, CA, USA) in a subset of animals that were evaluated through day 8 only. When the instrument indicated the proper frontal and diagonal alignment were obtained, 7 measurements were taken. Of the seven values collected from each eye, the highest and lowest values were excluded, and the mean of the remaining five values was reported.

**Histopathology**

On days 2, 8, 16, and 43 (Table), a subset of animals was anesthetized, using sodium pentobarbital and exsanguinated. Following enucleation, the bulbar conjunctivae were marked for orientation, and the eyes were fixed in a modified Davidson’s fixative for 48 to 96 hours, prior to transfer to 10% neutral-buffered formalin. The fixed eyes were trimmed.
### RESULTS

#### Aqueous Flare Response

Slit-lamp evaluations revealed a dose-related increase in aqueous flare beginning 4 hours post dose at level of \( \geq 0.05 \) EU/eye (Fig. 1A). This response peaked approximately on days 2 to 3 and reached an average of grade 1 to 2 in eyes given \( \geq 0.1 \) EU/eye. The aqueous flare resolved by day 5 in a subset of animals evaluated through day 8, although some additional trace levels of flare were observed between days 9 and 12 at \( \geq 0.1 \) EU/eye, potentially related to the peak vitreous cell response seen during this period in these dose groups (Fig. 2). Although variable, quantitative measurements of aqueous flare using LFP (expressed in photon counts per millisecond, ph/ms) generally agreed with the slit-lamp evaluation of aqueous flare, showing a dose-related response at \( \geq 0.05 \) EU/eye with a peak at 24 hours in all dose groups (Fig. 1B). Grade “0” in the slit-lamp examination correlated with a range of 2.8 to 142.6 ph/ms (mean \( \pm SD \), 30.3 ph/ms). Neither the slit-lamp examination nor the LFP was able to detect any evidence of aqueous flare in the 0.01 EU/eye dose group. Aqueous cell was first noted on day 3 in eyes given \( \geq 0.05 \) EU/eye with a peak onset of aqueous flare at this interval. In this subset of animals evaluated through day 8, ITV was administered in one eye and peaked on days 3 and 4 (Fig. 1C). The highest severity of aqueous cell response was observed in the 0.5 EU/eye was grade 4, and no evidence of aqueous cell response was observed in the 0.01 EU/eye dose group. Although the aqueous cell had resolved by day 8 in all eyes, trace levels were observed between days 9 and 12 in the 0.5 EU/eye group. This seemed to correlate with trace levels of aqueous flare during this period and might have been related to the peak vitreous cell response seen during this period in these dose groups (Fig. 2).

#### Aqueous Cell Response

Aqueous cell was first noted on day 3 in eyes given \( \geq 0.05 \) EU/eye and peaked on days 3 and 4 (Fig. 1C). The highest severity observed in the 0.5 EU/eye was grade 4, and no evidence of aqueous cell response was observed in the 0.01 EU/eye dose group. Although the aqueous cell had resolved by day 8 in all eyes, trace levels were observed between days 9 and 12 in the 0.5 EU/eye group. This seemed to correlate with trace levels of aqueous flare during this period and might have been related to the peak vitreous cell response seen during this period in these dose groups (Fig. 2).
to the strong vitreal cell response observed during this period of time in these dose groups.

**Vitreal Cell Response**

The vitreal cell response followed a comparable but delayed time course from that of the aqueous response. No vitreal cell response was observed in the 0.01 EU/eye group. The first trace evidence of vitreal cells was observed on day 5, and by day 8, a dose-dependent vitreal cell was observed in eyes given ≥0.05 EU/eye in animals evaluated through day 8 (Fig. 2A). Time course of vitreal cell response was further characterized over 43 days (Fig. 2B). In that subset of animals, dose at 0.05 EU/eye confirmed previous response, and that dose level produced a moderate grade 2+ response that peaked on day 9. At ≥0.5 EU/eye, a grade response range of 3 to 4+ was first
observed on day 3 and peaked on day 9. Dose at $\geq 0.5$ EU/eye produced a severe response grade up to 4+ over days 9 to 12, decreasing in severity starting on day 16. The mild response ranging from trace to grade 1+ persisted for an extended period of time for dose of $\geq 0.05$ EU/eye, nearly but not completely resolving by day 43 (Fig. 2B).

**Other Clinical Responses**

Vitreal haze at 0.1 EU/eye, incomplete pupil dilation, white vitreal floaters, mild to moderate degraded view of fundus, and hyperemic optic disc at $\geq 0.5$ EU/eye were noted on day 2 or 3. Mild conjunctival hyperemia and a mildly degraded view of the fundus were noted in $\geq 0.1$ EU/eye on day 3. These findings

**FIGURE 2.** The vitreal cell response observed following intravitreal endotoxin administration was delayed and dose-related, showing increase by day 8 ([A] results are mean ± SEM). Time course of vitreal cell response was further characterized over 43 days ([B] results are mean ± SEM). Overall, the vitreal cell response was more durable than the aqueous response. Vitreal cell response was largely resolved by day 43 (see Table for the number of eyes [no. of animals] per each time point).

**FIGURE 3.** A trend toward decreased intraocular pressure was observed in the higher dose groups between 24 and 72 hours post dose. This trend was not statistically significant at any time point but was consistent with the inflammatory reaction within the eye. Results are mean ± SD (see Table for the number of eyes [no. of animals] per each time point).
completely resolved by day 4. Although not statistically significant, there appeared to be a slight decrease in mean IOP in eyes administered 0.1 EU/eye at 48 and/or 72 hours post dose, which corresponded with an increase in aqueous cell (Fig. 3). Such a reduction in IOP is consistent with an intraocular inflammatory response. Numerous procedure-related findings were observed, including conjunctival congestion, conjunctival swelling, conjunctival discharge, and clinical observations of red conjunctiva, squinting, corneal erosion (from drying of corneal surface), and vitreal floaters. These findings were sporadic and lacked a dose-response, so they were not considered related to the endotoxin administration.

**Ocular Pathology**

Microscopy findings of infiltrates of minimal mixed inflammatory cells in the vitreous and subconjunctiva and proteinaceous fluid in the anterior chamber and/or vitreous were observed in eyes given ≥0.1 EU/eye at day-2 interim necropsy (Fig. 4). At the day-8 interim necropsy, infiltrates of mononuclear cells with mild severity in the vitreous were observed in eyes given ≥0.01 EU/eye and proteinaceous fluid in the vitreous in eyes given ≥0.2 EU/eye. By day-16 interim necropsy, histopathologic changes were subtle and limited to minimal or mild mixed (although predominantly lymphocytic) infiltrates within the vitreous at dose of ≥0.1 EU/eye. In the 0.2 EU/eye, some proteinaceous fluid was also apparent within the vitreous. Inflammation and/or morphologic changes were not observed elsewhere in the eye. By day-43 terminal necropsy at all doses, inflammation within the vitreous had decreased (with infiltrates consisting exclusively of mononuclear cells) or, in some animals, resolved.

**DISCUSSION**

Bacterial endotoxin is a common contaminant of therapeutic formulations and surgical instruments and devices. Possible sources of contamination include water, raw materials that contain lipopeptides, peptidoglycans, or other proteins.18–20 Due to endotoxin heat resistance, it is difficult to remove from contaminated products and is therefore tightly controlled and analyzed in most products intended for injection or implantation in humans. Several studies have demonstrated that the eye is uniquely sensitive to the effects of endotoxin contamination.4,5 However, much of this work has focused on anterior segment and intraocular routes of administration of endotoxin-laden formulations or OVDs. Contamination following ITV injection of therapeutic agents is of concern due to the recent increase of intravitreal therapies. In addition, certain biologic therapies may be produced in *E. coli*, a gram-negative bacteria. Ophthalmic viscosurgical devices are also commonly used in the posterior segment, and numerous surgical procedures expose the vitreous to a variety of surgical tools. In order to assess the risk associated with endotoxin contamination of ITV formulations or devices, a more thorough understanding of the effects of this contaminant in this segment is critical.

The current study was designed to define the response to ITV injection of a reference, purified endotoxin and to define a NOEL for this effect in rabbits. Our results demonstrated a rapid dose-related anterior chamber reaction beginning with aqueous flare as early as 4 hours post dose and aqueous cell within 48 hours post dose (Fig. 1). The rapid time course of the reaction is in line with data published by several other groups following both intracameral and intravitreal endotoxin injections.5,4,7,21
In vehicle-treated eyes, a single eye was noted with grade 2+ aqueous flare at 24 hours post dose, consistent with the peak onset of aqueous flare in eyes treated with endotoxin. A high amount of flare using LFP (359.1 ph/ms) confirmed the clinical finding of aqueous flare at this interval. Although it was noted only in 1 of 29 eyes, it does show that the intravitreal dosing procedure, on rare occasions, may induce anterior segment inflammation. Interestingly, the aqueous cell response was slightly more rapid and severe in the subset of animals evaluated through day 43, with some animals in that arm reaching response grades 3 to 4+ at 48 hours, whereas no animal exceeded grade 2+ in the subset of animals evaluated through day 8 only. This discrepancy is likely due to interanimal variability.

A more delayed posterior segment response characterized by vitreal cell was observed beginning on day 5, and by day 8, a dose-dependent vitreal cell was observed in animals given ≥0.05 EU/eye and evaluated through day 8 (Fig. 2A). Because vitreal cell response started to increase by day 8, the posterior segment inflammatory response was further characterized over 45 days (Fig. 2B). Surprisingly, the onset of vitreal cell response at ≥0.5 EU/eye level was observed as early as day 3, with a response grade ranging from 3 to 4+, peaking on day 9, and decreasing starting day 16 (Fig. 2B). The temporal difference and magnitude of vitreal cell response between the two subsets of animals at a common dose of 0.5 EU/eye potentially related to the peak anterior chamber response seen during this period (Fig. 1) and may highlight the interanimal variability. Interestingly, vitreal cell response persisted for more than 42 days at trace to 1+ levels in all dose groups (Fig. 2B) and was consistent with the identification of minimal inflammatory cell infiltrates histologically. The slow recovery of the vitreal cellular response has previously been described22 and suggests that, once present, inflammatory cells within the vitreous are cleared much more slowly than from the aqueous, perhaps due to the higher viscosity and lower fluid flow in vitreous. Although it is delayed, the severity and duration of this vitreous response may become the dose-limiting effect following ITy administration of endotoxin-contaminated products. In contrast, the aqueous response (flare and cell) was generally less severe and more transient. It is interesting that, despite administration of the endotoxin directly to the vitreal chamber, the initial response was observed within the aqueous. This may be related to clearance of the endotoxin and associated inflammatory proteins and cells through aqueous drainage.

This initial anterior reaction that resolved clinically within 5 to 8 days was associated with microscopy findings of infiltrates of minimal mixed inflammatory cells in the vitreous and subconjunctiva and proteinaceous fluid in the anterior chamber and/or vitreous on day-2 interim necropsy in eyes given ≥0.1 EU/eye (Fig. 4). On day-8 interim necropsy, infiltrates of mononuclear cells with mild severity in the vitreous at ≥0.01 EU/eye level and proteinaceous fluid in the vitreous at ≥0.2 EU/eye was observed (Fig. 4). The differences in the infiltrating cell population on day 2 (mixed) versus those on day 8 (mononuclear cell) were reflective of the normally expected time course of inflammatory cell infiltrates, wherein granulocytic cells arrive within hours and resolve within a few days if the stimulus is removed and mononuclear cells arrive after a few days. Mild mononuclear cell infiltrates were present in eyes receiving either 0.01 or 0.05 EU/eye on day-8 interim necropsy (but not on day-2 interim necropsy).

Sakimoto et al.3 used slit-lamp ophthalmoscopy and LFP to identify a NOEL of 0.23 EU/eye following intracameral administration of endotoxin. However, other groups have reported inflammatory response following intracameral injection of OVDs containing ≥0.02 EU/eye levels of endotoxin.4,5 In the current study, local ocular effects of ITy endotoxin were assessed using slit-lamp biomicroscopy, indirect ophthalmoscopy, laser flare meter measurement, and histopathology over the course of 43 days, and we found the NOEL for ITy endotoxin to be 0.01 EU/eye, suggesting that the vitreal cavity is more sensitive to the effects of endotoxin than the anterior segment and aqueous chamber. This may be related to the more rapid turnover of aqueous humor than the slow turnover of vitreous humor as mentioned above, which likely leads to a longer residence time of endotoxin within the eye.

In conclusion, we have defined the dose-response of intravitreal endotoxin in anterior and posterior segments of Dutch Belted rabbits. A NOEL of 0.01 EU/eye and a lowest observed effect level (LOEL) of 0.05 EU/eye were observed, suggesting that the vitreal cavity is approximately 2- to 10-fold more sensitive to endotoxin administration than the anterior chamber. Importantly, these levels are well below the levels reported in a survey of endotoxin levels of OVDs23 and, therefore, may be clinically relevant. Current regulatory guides vary as to the acceptable levels of endotoxin. For example, the US Food and Drug Administration set a limit of ≤0.2 EU/device for single-use intraocular ophthalmic devices.24 A limit of endotoxin for parenterally administered products is defined on the basis of dose, expressed as K/M, where K is 5 EU/kg for any route of administration other than intrathecal injection (for which K is 0.2 EU/kg), and M represents maximum recommended bolus dose in a single hour period.25 Although our data demonstrate an effect of significantly lower levels of endotoxin in the rabbit eye, it should be noted that the translatability of these findings to humans is unknown at this time. Although suspected cases of sterile uveitis related to endotoxin contamination in humans have been reported,14-16,26 the exact endotoxin levels that are responsible for these effects are generally unknown, and it is possible that humans are less sensitive to the effects of ocular endotoxin contamination. Additional research is required to better understand the relevance of these data for human risk assessment. It is also important to note that reference standard endotoxin preparations, although suitable for analytical calibration standards of the bacterial endotoxin test and as a spike in recovery studies, bear minimal resemblance to the real world endotoxin contamination that may be present during biotechnology/pharmaceutical manufacturing.20 However, these results highlight the importance of regular endotoxin testing for all products intended for intravitreal administration. Certainly, when using rabbits as a test species in order to evaluate local ocular tolerability and distinguish contribution from test article, endotoxin levels of intravitreally administered test articles should be kept at ≤0.01 EU/eye for both scientific and animal welfare reasons.

**Acknowledgments**

Disclosure: V. Bantseev, Genentech, Inc. (E); P.E. Miller, None; E. Bentley, None; C. Schuetz, Genentech, Inc. (E); T.M. Streit, Covance Laboratories, Inc. (E); B.J. Christian, Covance Laboratories, Inc. (E); C. Farman, None; H. Boiler, Genentech, Inc. (E); E.A. Thackaberry, Genentech, Inc. (E)

References

3. Sakimoto A, Sawa M, Oshida T, Sugaya T, Hirono T, Ishimori A. Minimum endotoxin concentration causing inflammation in...


