

Comparing the Effectiveness of Intravitreal Levofloxacin and Ceftazidime in Experimental *Pseudomonas aeruginosa* Endophthalmitis

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Received: February 06, 2018; **Published:** March 03, 2018

Abstract

Purpose: To evaluate the effectiveness of intravitreal 0.5% levofloxacin as an alternative treatment for *Pseudomonas aeruginosa* endophthalmitis in an experimental model.

Design: A randomized, masked, controlled-experimental study was conducted from August to September 2015 at animal laboratory of Biomedical Research Centre for Health Research and Development Agency, National Center General Hospital Dr. Cipto Mangunkusumo (RSCM).

Methods: Twelve New Zealand white rabbits were divided evenly into two groups. The vitreous cavities of their right eyes were inoculated with 2×10^5 CFU/0.1 mL of *Pseudomonas aeruginosa* suspension. Afterwards, group A was treated with intravitreal 0.5% levofloxacin while group B received intravitreal injection of 2.25 mg/0.1 mL ceftazidime 24 hours after bacterial inoculation. The clinical evaluations of the eyes in each group were performed daily from day 1 to 6 after inoculation. Microbiological and histopathological examinations were evaluated on day 6 after inoculation.

Results: The mean clinical assessment scores 24 hours after inoculation were not statistically different ($p > 0.05$). The clinical score at day 1 was not found to be significantly different from the score at day 6. Two rabbits experienced improvements in the levofloxacin group but these were not significant. The number of microbiological bacteria in group A and group B decreased to 1.5×10^2 ($4 \times 10^1 - 7.3 \times 10^3$) CFU/0.1 mL; however, microbiological analysis and histopathological scoring showed no significant difference between group A and B (for each, $p > 0.05$).

Conclusion: Intravitreal 0.5% levofloxacin ophthalmic appears to be effective in treating *Pseudomonas aeruginosa* endophthalmitis in rabbits, although not superior to intravitreal ceftazidime administration. Therefore, intravitreal 0.5% levofloxacin may be used as an alternative to ceftazidime to treat *Pseudomonas aeruginosa* endophthalmitis.

Keywords: Ceftazidime; Endophthalmitis; Levofloxacin; *pseudomonas aeruginosa*

Abbreviations

MIC90: Minimum Inhibitory Concentration Required to Inhibit the Growth of 90% of Organisms; RSCM: Rumah Sakit Dr. Cipto Mangunkusumo/National Center General Hospital Dr. Cipto Mangunkusumo

Introduction

Endophthalmitis is an inflammation involving intraocular tissue and fluid that can be caused by microbes including bacteria, fungi or parasites which occupy the anterior and posterior segments of the eye [1]. Endophthalmitis is an ophthalmological emergency and may lead to permanent visual deterioration or even blindness if not dealt with prompt therapy [2].

A retrospective study conducted in Japan reported 24.7% incidence of endophthalmitis between January 2007 and July 2010 [3]. At RSCM, the incidence of postoperative intraocular endophthalmitis was 0.45% and 74.7% of all endophthalmitis cases were found after cataract extraction [4]. *Pseudomonas sp.* was the most common etiology of postoperative endophthalmitis found in RSCM [4]. Endophthalmitis due to *Pseudomonas sp.* has a poor visual prognosis. Pinna., *et al.* reported 10 out of 20 eyes with *Pseudomonas* endophthalmitis undergoing evisceration [5].

The gold standard treatment of bacterial endophthalmitis is vitrectomy. However, if it could not be performed immediately, intravitreal antibiotics may be used as an alternative therapy [1,6,7]. Standard intravitreal antibiotics, such as vancomycin for gram positive microorganism and ceftazidime for gram negative microorganism, should be given [7,8]. However, ceftazidime is not commercially available in the appropriate therapeutic dose for intravitreal injection, thus requiring manual dilution. This may increase the risk of contamination and dilution errors, which may further cause intraocular toxicity. In India, the sensitivity of gram negative bacteria against amikacin and ceftazidime was only 68% and 63% [9].

Levofloxacin is a third generation of fluoroquinolone, which eliminates broad spectrum of gram positive and negative ocular pathogen bacteria. Levofloxacin acts by inhibiting bacterial topoisomerase IV and DNA gyrase (enzymes that are required for DNA replication, transcription, repair, and recombination) [10-12].

Levofloxacin 0.5% ophthalmic solution is preservative-free and its pH is lower than normal rabbit vitreous, hence it is safer to be injected intravitreally [13]. The appropriate dose of intravitreal levofloxacin which does not cause retinal toxicity in rabbit eyes is 500 - 625 µg and this dose is above the Minimum Inhibitory Concentration (MIC) 90 values of ocular pathogens that may cause endophthalmitis [14-16]. Thus, this study aimed to compare the efficacy of levofloxacin 0.5% with intravitreal ceftazidime in *Pseudomonas aeruginosa* endophthalmitis.

Material and Methods

This research used experimental design in which blinding and randomization were applied to divide the animal subjects into two groups. This research was conducted in the animal laboratory of Biomedical Research Centre for Health Research and Development Agency, National Center General Hospital dr. Cipto Mangunkusumo (RSCM) from August to September 2015.

Animal models

Twelve New-Zealand white rabbits, weighing 2 - 3 kg and aged 3 - 4 months, were maintained in accordance with institutional guidelines and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmologic and Vision Research. The rabbits were divided into two groups evenly. All treatments and surgeries were performed under general anesthesia with intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). Topical anesthesia was achieved by tetracaine hydrochloride 0.5% (Cendo Pantocain, Bandung, Indonesia). The rabbit pupils were dilated with phenylephrine hydrochloride 2.5% (Cendo Efrisel, Bandung, Indonesia) and tropicamide 1% (Cendo Midriasil, Bandung, Indonesia).

Experimental Design (Inoculation and Intravitreal Injection)

Standard isolates of *Pseudomonas aeruginosa* ATCC 27853 were used in this study. The bacteria were proven sensitive to levofloxacin and ceftazidime using Kirby-Bauer disc diffusion susceptibility testing. Then, the harvested bacteria were made into suspension equal to 0.5 McFarland standard. This suspension of *P. aeruginosa* was adjusted by serial dilution in sterile physiological saline to obtain a final concentration of approximately 2×10^5 CFU/ml to induce endophthalmitis by intravitreal injection.

All intravitreal injections were performed by a single surgeon using a 1 mL tuberculin syringe with a 30-gauge needle and inserted 2 mm posterior to the limbus in the superotemporal quadrant. Previously, anterior paracentesis (0.1 ml) was performed to avoid elevation of intraocular pressure. Twenty-four hours after intravitreal inoculation of *P. aeruginosa*, the eyes were confirmed to exhibit clinical signs of endophthalmitis and were randomly assigned into two groups. Eyes with hyphema or vitreous hemorrhage were excluded. The drop out criteria were death, illness during the study, or any microbiological examination showing microorganism other than *P. aeruginosa*.

The right eyes of rabbits in group A received intravitreal 0,5%/0,1 mL levofloxacin injection, whereas those in group B received intravitreal 2,25 mg/0,1 ml Ceftazidime injection. The rabbits were examined before and after injection using slit lamp (Nidek SL-1600) and indirect ophthalmoscope (Heine BIO Omega 500).

Clinical Evaluation

A masked observer examined the eyes of rabbits in each group daily from the 1st until 6th day after inoculation using slit lamp and indirect ophthalmoscope. Clinical examination scores were graded according to scale as shown in table 1.

| Findings | Score | | | | |
|-------------------------|-------|----------------------|----------------------------------|--------------------------------|-----------------------|
| | 0 | 1 | 2 | 3 | 4 |
| Cornea | | | | | |
| Transparency | Clear | Mild | Moderate (iris visible) | Severe | Opaque |
| Vessels | None | < 1 mm | 1 - 2 mm from limbus | 3 - 4 mm from limbus | Central involvement |
| Abscess (size) | None | < 1 mm | 1 -3 mm | 3 - 4 mm | > 5 mm |
| Anterior Chamber | | | | | |
| Protein flare | None | Trace | Mild | Moderate | Severe |
| Inflammatory cells | None | Trace | Mild | Moderate | Severe |
| Fibrin/hypopyon | None | Mild Strand | Moderate Strands, < 15% hypopyon | Severe Strands, < 15% hypopyon | No iris detail |
| Hyphema | None | Mild (< 25%) | Moderate (25 - 50%) | > 50% | 8-ball (full hyphema) |
| Iris | | | | | |
| Blood vessels | None | Mild | Moderate | severe | Neovascularization |
| Vitreous | | | | | |
| Protein flare | None | Trace | Mild | Moderate | Severe |
| Opacities | None | Cells | Multiple clumps | Red reflex | Totally opaque |
| Retinal detachment* | None | 25% | 50% | 75% | Total |
| Optical media | Clear | Vessels outline only | Sharp red reflex | Dull red reflex | Totally opaque |

Table 1: Clinical grading scale [16].

Microbiological Examination

After the last ophthalmic examination at day 6, 0.2 mL vitreous aspirates were obtained for microbiological analysis. Samples of the aspirates were serially diluted and plated for quantification on Total Plate Count (TPC) medium and incubated at 37oC for 24 hours. After incubation, surface colonies were counted and identified as *P. aeruginosa*. The microbiological examination was performed by masked analyst.

Statistical Analysis

The data were analyzed by SPSS 11.0 software. T-test and Mann-Whitney U test were used in statistical analysis based on normality distribution and $p < 0.05$ was considered as statistically significant.

Results and Discussion

Results

Endophthalmitis developed within 24 hours after inoculation and was characterized by corneal edema and opacities, inflammatory reaction of the anterior chamber, fibrin and vitreous opacities. The baseline characteristics of subjects are mentioned on table 2.

| Characteristics | Group A (levofloxacin 0,5%) | Group B (ceftazidime 2,25 mg/0,1 mL) | p |
|--|-----------------------------|--------------------------------------|------|
| Rabbits (n) | 6 | 6 | |
| Body weight (mean) g | 2800 ± 90.83 | 2740 ± 92.89 | 0.28 |
| Total clinical scores on 24 hours after inoculation (mean) | 22.50 ± 2.80 | 24.00 ± 2.36 | 0.35 |

Table 2: Baseline characteristic of subjects.

Figure 1 shows higher scores on 3rd day after inoculation in the both groups. There was some improvement in the clinical score of the two groups after the third day, especially in the levofloxacin group.

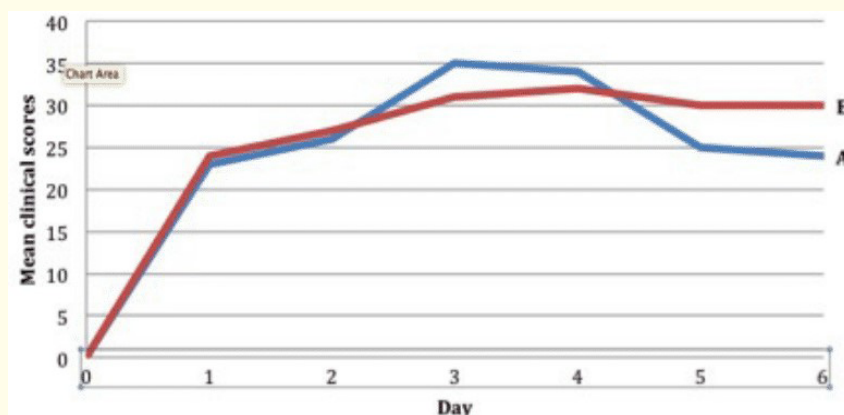


Figure 1: Daily mean clinical scores changes.

Table 3 shows improvement which occurs in two animal models in the levofloxacin group with $p = 0.455$.

| Improvement | Levofloxacin | Ceftazidime | p |
|-------------|--------------|-------------|------|
| Yes | 2 | 0 | 0.45 |
| No | 4 | 6 | |

Table 3: Clinical Improvement.

Clinical assessment scores on day 6 and Δ scores between two groups, as shown in table 4, both show no statistically significant differences ($p > 0.05$).

| Group | Day 1 | p | Day 6 | p | Δ scores | p |
|---------|--------------|------|--------------|------|-------------|------|
| Group A | 22,50 ± 2,80 | 0,35 | 27,50 (2-32) | 0,46 | 5 (-16 - 8) | 0,52 |
| Group B | 24,00 ± 2,36 | | 29,83 ± 5,45 | | 5,83 ± 5,15 | |

Table 4: Clinical scores comparison and score difference between day 1 and 6.

Figure 2 shows that clinical improvement occurs after day 3 in both groups.

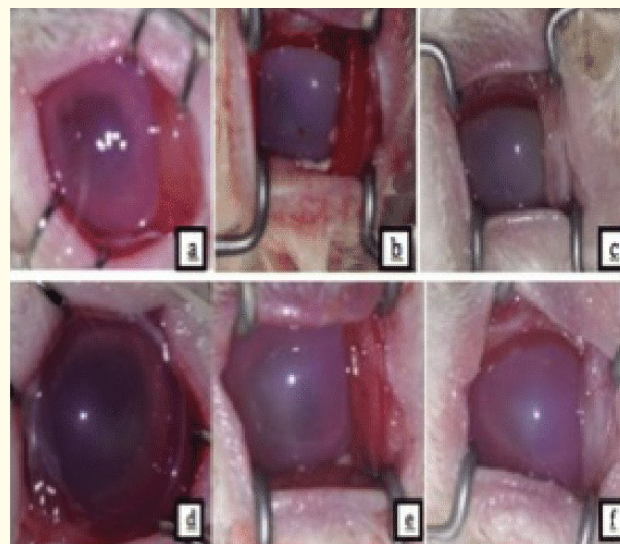


Figure 2: Clinical evaluation of day 1, 3, and 6 on both groups; group A (a, b and c) and group B (d, e and f).

The number of bacterial colonies (Table 5) and histopathological grading score (Table 6) between both groups show no significant difference. The result of histopathological examination is presented in figure 3.

| Group | CFU/0,1 mL | p |
|-----------------------------|--|------|
| Group A (levofloxacin 0,5%) | 1,5 x 10 ² (6,8 x 10 ¹ - 1,5 x 10 ²) | 1,00 |
| Group B (ceftazidime) | 1,5 x 10 ² (4 x 10 ¹ - 7,3 x 10 ³) | |

Table 5: Bacterial count on day 6.

| Group | Total scores | p |
|---------|--------------|------|
| Group A | 13,50 ± 5,32 | 0,68 |
| Group B | 15,50 ± 1,97 | |

Table 6: Histopathological grading on day 6.

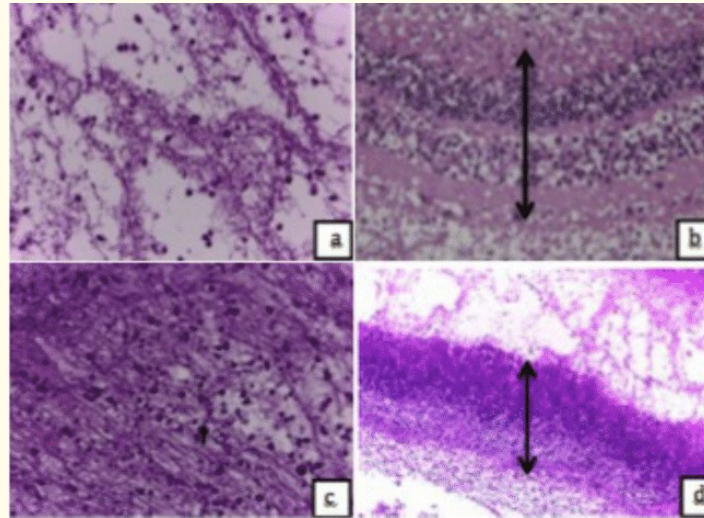


Figure 3: Group A (a and b) with moderate histopathological scores in vitreous and retinal tissue; (b) Arrow showing retinal thickening and moderate inflammatory cells (original magnification, 40x); Group B (c and d) with high histopathological scores; (c) Arrow showed vitreous cavity filled with neutrophil infiltration (original magnification, 40x); (d) Arrow showed no discernible retinal layers (original magnification, 40x).

Discussion

Endophthalmitis is a rare but sight-threatening disease [2]. *Pseudomonas* is the most common cause of endophthalmitis in RSCM with poor visual prognosis [4]. *Pseudomonas* infections are invasive and toxigenic [18-21]. This study used intravitreal levofloxacin 0.5% injection. Levofloxacin is widely used in the treatment of eye infections. A study conducted by Aziza, *et al.* showed intravitreal injections of levofloxacin 0.5% ophthalmic solution effectively improved clinical outcomes and reduced bacterial colonies compared to control group [22].

The results in this study showed that clinical scores of both group on day 6, as well as the mean difference of day 1 and day 6 did not have a statistically significant difference. However, intravitreal injection of 0.5% levofloxacin ophthalmic solution was able to reduce the clinical inflammation scores in the anterior and posterior segment after the third day of inoculation. On day 6, the clinical assessment score of levofloxacin group tends to decrease more compared to the ceftazidime group.

The results of bacterial counting in both group was not significantly different. It is also similar to study conducted by Ferrer, *et al* [23]. The study showed the effectivity of levofloxacin 1.5% in bacterial endophthalmitis, which clinically decreased the inflammation and the number of bacteria colonies, but did not show a statistically significant difference when compared with vancomycin therapy.

Further microbiological examination showed that colonies of bacteria did not grow on one of the eyes of group A. The score of vitreous histopathological was zero and this particular rabbit showed clinical improvement.

The aim of histopathological examination is to evaluate the intraocular tissue damage and indicate the prognosis of visual function. Microscopical examination showed that the total histopathologic score was not different in both groups.

In both groups, most of the subject animals showed severe inflammation with score more than 13 due to the invasive and toxigenic nature of *Pseudomonas aeruginosa* infections. *Pseudomonas* can invade and proliferate within the eye in an acute manner and produce extracellular enzyme that can further increase bacterial invasion, cause host tissue destruction, and cell lysis [18-21].

The histopathologic score of retina in most samples was 3. This condition is similar to the study conducted by Aziza, *et al.* in which 8 out of 13 eyes in levofloxacin group had histopathological score of 3 or more [22]. In this study there was no significant difference ($p > 0.05$) in histopathological examination mean scores between the two groups.

Conclusion

The effectivity of intravitreal levofloxacin 0.5% ophthalmic solution 0.1 mL injection was similar to ceftazidime and thus levofloxacin can be used as an alternative antibiotic in the treatment of mild and moderate endophthalmitis due to *Pseudomonas aeruginosa*.

Acknowledgement

Address for reprints and sources of support that require acknowledgement is none.

Conflict of Interest

There is no conflict of interest in this study.

Bibliography

1. "Endophthalmitis". In: American Academy of Ophthalmology. Intraocular inflammation and uveitis. 2010-2011. Section 8 Basic and Clinical Science Course. San Francisco: American Academy of Ophthalmology (2009): 269-80.
2. Cabezas CL, *et al.* "Antibiotics in endophthalmitis: microbiological and pharmacokinetic considerations". *Current Clinical Pharmacology* 5.1 (2010): 47-54.
3. Shimizu Y, *et al.* "Prevalence of drug resistance and culture-positive rate among microorganisms isolated from patients with ocular infections over a 4-year period". *Clinical Ophthalmology* 7 (2013): 695-702.
4. Aziza Y and Susiyanti M. "Incidence and Influencing Factors of the Management's Result of Post Intraocular Surgery Endophthalmitis at Cipto Mangunkusumo Hospital within January 2007 – July 2010". Jakarta: Ophthalmology Department Medical Faculty University of Indonesia - Cipto Mangunkusumo Hospital (2010).
5. Pinna A, *et al.* "An outbreak of post-cataract surgery endophthalmitis caused by pseudomonas aeruginosa". *Ophthalmology* 116.12 (2009): 2321-2326.
6. Barry P, *et al.* "ESCRS guidelines on prevention, investigation, and management of post-operative endophthalmitis". Santen (2007): 1-28.
7. Eichenbaum DA, *et al.* "Postoperative endoftalmitis". In: Steinert RF, Chang DF, Miyajima HB, Gimbel HV, Koch DD, Lane SS, editors. Cataract surgery. Duxbury: Saunders (2010): 671-678.
8. Endophthalmitis Vitrectomy Study Group. "Results of the endophthalmitis vitrectomy study: a randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis". *Archives of Ophthalmology* 113.12 (1995): 1479-1496.
9. Elfrigg CW, *et al.* "Endophthalmitis caused by pseudomonas aeruginosa". *Ophthalmology* 110.9 (2003): 1714-1717.
10. Blondeau JM. "Fluoroquinolones: mechanism of action, classification, and development of resistance". *Survey of Ophthalmology* 49.2 (2004): 73-78.

11. Levofloxacin (2007).
12. Hwang DG. "Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones". *Survey of Ophthalmology* 49.2 (2004): S79-S83.
13. Santen. Quixin (levofloxacin ophthalmic solution) 0.5%: prescribing information (2008).
14. Gurler B., et al. "A study on toxicity of intravitreal levofloxacin in rabbit". *Current Eye Research* 24.4 (2002): 253-262.
15. Kazi AA., et al. "Intravitreal toxicity of levofloxacin and gatifloxacin". *Ophthalmic Surgery, Lasers and Imaging Retina* 37.3 (2006): 224-229.
16. Ryan EH and Mizener JB. "Tissue plasminogen activator and staphylococcus epidermidis endophthalmitis". *Archives of Ophthalmology* 111.8 (1993): 1117-1122.
17. Meredith TA., et al. "Spontaneous sterilization in experimental staphylococcus epidermidis endophthalmitis". *Investigative Ophthalmology and Visual Science* 31.1 (1990): 181-186.
18. Pier GB. "Pseudomonas and related gram negative bacillary infections". In: Goldman L, Schafer AI. Goldman's cecil medicine. Twenty-fourth edition. Philadelphia: Saunders (2012): 1877-1881.
19. Todar K. "Pseudomonas aeruginosa". University of Wisconsin - Madison Department of Bacteriology (2011).
20. Gellatly SL and Hancock REW. "Minireview pseudomonas aeruginosa: new insights into pathogenesis and host defenses". Dalam: Bavoil P. Pathogens and diseases. Vancouver: Blackwell publishing (2013): 159-173.
21. Pseudomonas aeruginosa (2015).
22. Aziza Y., et al. "Efficacy of intravitreal levofloxacin 0,5% ophthalmic solution in treating pseudomonas aeruginosa endophthalmitis on a rabbit model". *Asia-Pacific Academy of Ophthalmology* 2.3 (2013): 199-205.
23. Ferrer C., et al. "Bactericidal effect of intravitreal levofloxacin in an experimental model of endophthalmitis". *British Journal of Ophthalmology* 92.5 (2008): 678-682.

Volume 9 Issue 4 April 2018

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