

The Storage Bioefficacy of Vancomycin, Amikacin, Ceftazidime at Various Time-Points and Temperatures: Antibiotics Used for Intravitreal Injections

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Received: September 03, 2018; Published: October 30, 2018

Abstract

Purpose: We evaluated bioactive efficacy of vancomycin, ceftazidime and amikacin stored at five temperatures (room, -20°C, -80°C, 6°C, 37°C) and four time points (day 0, 30, 90, 180) to establish long-term storage of antibiotics for endophthalmitis.

Methods: Vancomycin (1.0 mg/0.1 ml), ceftazidime (2.25 mg/0.1 ml) and amikacin (0.4 mg/0.1ml) were reconstituted to match dose concentrations used in intravitreal injections. Each drug, for each temperature and time point, was serially diluted using minimum inhibitory concentration (MIC) testing and inoculated with *Staphylococcus aureus* (ATCC 29213) (SA). After 24 hours of incubation, MIC was determined with the lowest dilution that produced growth. The dilution corresponding with SA MIC was the outcome measure for bioactivity.

Results: An arbitrary minimum of 90% bioactivity was chosen for acceptable stability. Vancomycin was stable for all time points at 6, -20°C and -80°C; less stable for room temperature at 180 days (9.4% decrease), 37°C at 90 days (33.1% decrease), and 37°C at 180 days (46.3% decrease). Ceftazidime was stable at 30 days at -80, -20 and 6°C; stable at -20°C at 90 days; less stable at 30 days at room temperature (26.03% decrease) and at 37°C (100% decrease). Amikacin was stable for all time points at -80°C and -20°C; less stable at 180 days at 6°C and room temperature (12% decrease) and at 30 days and 180 days at 37°C (10% decrease).

Conclusion: Temperature and time can affect stability of intravitreal antibiotics for endophthalmitis.

Keywords: Intravitreal Antibiotics; Endophthalmitis; Off-Label Antibiotics; Ophthalmic Antibiotics; Vancomycin; Amikacin; Ceftazidime

Abbreviations

MIC: Minimum Inhibitory Concentration; SA: *Staphylococcus aureus*; EVS: Endophthalmitis Vitrectomy Study; FDA: Food and Drug Administration; TSB: Trypticase Soy Broth

Introduction

Endophthalmitis is a serious complication of ocular surgery and intravitreal injections, and can lead to severe visual impairment. In fact, endophthalmitis from intravitreal injections are suggested to have even poorer visual outcomes when compared with endophthalmitis following cataract surgery [1]. Therefore, immediate intervention and treatment is necessary to preserve vision

Treatment for endophthalmitis has increasingly favored the use of intravitreal antibiotics and have now become the mainstay of treatment [2]. The Endophthalmitis Vitrectomy Study (EVS) was a randomized clinical trial that showed similar visual outcomes after intravitreal injection of antibiotics with or without pars plana vitrectomy in patients with endophthalmitis within 6 weeks of cataract surgery or lens exchange with a presenting visual acuity of hand motion or better; therefore, validating the need for prompt intravitreal antibiotics. According to EVS, 94.2% of culture-confirmed cases involved Gram-positive bacteria [3]. In order to cover these bacteria and Gram-negative organisms, current recommendations for empirical treatment are vancomycin 1.0 mg/0.1 mL and ceftazidime 2.25 mg/0.1 mL² or amikacin 0.4 mg/0.1 mL.

Many intravitreal antibiotics are now obtained via compounding pharmacies and are considered off-label uses of these antibiotics with no official standard from the Food and Drug Administration (FDA), including storage recommendations. Given the high acuity nature of the disease, accessibility and storage for these prepared intravitreal injections is crucial for the prompt treatment of endophthalmitis. A prior study by Mehta, et al. [4] tested antibiotic stability using a disk diffusion method to measure biologic activity; however this method does not allow for precise zone of inhibition measurements less than one millimeter.

Instead, this study utilized a dilution method to more precisely measure the biologic activity of vancomycin, ceftazidime and amikacin under varied conditions of time (time 0, 30 days, 90 days, 180 days) and storage temperatures (room temperature, -20°C, -80°C, 6°C and 37°C).

Materials and Methods

Preparation of Antibiotics

The most common antibiotic concentration used in intravitreal injections are the following: vancomycin at 1.0 mg/0.1 mL, ceftazidime at 2.25 mg/0.1 mL and amikacin at 0.4 mg/0.1 mL [3]. Each antibiotic was reconstituted in sterile water. The vancomycin solution was prepared by adding 10 mL of sterile water to 1g of vancomycin (Hospira, Inc. Lake Forest, IL) to get a 100 mg/mL solution, of which 1.11 mL was added to another 10 mL of sterile water to get 10 mg/mL concentration. The ceftazidime solution was prepared by adding 10 mL of sterile water to 1g of ceftazidime (GlaxoSmithKline, RTC, NC 27709) to get a 100 mg/mL solution, of which 2.9 mL was added to another 10 mL of sterile water to get 22.5 mg/mL concentration. The amikacin solution was prepared by adding 10 mL of sterile water to 0.163 mL of amikacin solution of 250 mg/mL (Teva Parenteral Medicines, Inc, Irvine, CA) to get a 4 mg/mL concentration. The antibiotics were then placed in a separate vial for each time point so that only one vial was thawed for the appropriate time point that the experiment was conducted. The antibiotics were placed in dark boxes.

Staphylococcus aureus Inoculum

Standard quality control reference strain of *Staphylococcus aureus* (ATCC 29213) (SA) was used as the inoculum. SA was grown overnight at 37°C. The next day, SA was suspended into 5 mL of Trypticase Soy Broth (TSB) until there was slight turbidity to match 0.5 McFarland Standard for turbidity. Of the 5 mL of bacteria suspension, 100 microliters was added to 10 mL of Mueller-Hinton broth, which then was used to inoculate the wells with diluted antibiotic solution as described below. This was repeated at each time point that the experiment was conducted so that the bacteria was grown each time.

Experimental design

The following experiment was carried out in 96 well plates. A separate vial of each antibiotic was thawed for each time point that the experiment was conducted, so as to not disrupt the other antibiotic samples used at various time points. Each drug at the following temperatures: room temperature, -20°C (non frost-free freezer, Lab-Line Instruments, Inc., Melrose Park, IL), -80°C (Thermo-Fisher Scientific, Asheville, NC), 6°C and 37°C at time 0 was plated starting with 10 microliters and diluted by 10:1 from one well to the next well for up to 6 (10X) dilutions. Then each antibiotic from the 10:3 dilution well (the appropriate MIC was known to be in between the -3 and -4 dilution) was further diluted (0.1, 0.2, 0.4, 0.6, 0.8) for five dilutions to more precisely determine the dilution for determining the MIC. The bacterial suspension as described above was then added to the initial diluted wells at 90 microliters per well and then added to the second dilution set at 100 microliters per well. The plates were incubated at 37°C overnight. At 24 hours after inoculations, the plates were read for obvious turbidity and to determine the well with no turbidity at the lowest dilution to see which dilution was the most efficacious. Each antibiotic was plated in duplicate in the same 96-well plate. The above experiment was repeated at the 30, 60 and 180 day time points. The entire experiment was then repeated over the course of another 180 days in the exact fashion to ensure reproducibility. The percent differences in bioefficacy were calculated as the change in dilution between the various time point and time zero for each antibiotic at each temperature, divided by the dilution factor at time zero. The results presented below are averages of both the original and repeat experiment as the data from the two experiments were very similar.

Results

An arbitrary minimum of 90% bioefficacy was chosen for acceptable stability. A 10% decrease or greater in bioactivity was considered potentially problematic.

Vancomycin was stable at all time points for the following temperatures: -80°C, -20°C, 6°C and room temperature (9.4% decrease in bioefficacy at 180 days at room temperature). Vancomycin lost its efficacy at the 90 days at the 37°C with a 33.1% decrease in efficacy and by 180 days at that temperature, it showed a 46.3% decrease.

Ceftazidime was stable only up to 30 days at the following temperatures: -80°C, -20°C, and 6°C. At these storage temperatures, by 90 days, ceftazidime had greater than 10% decrease in bioefficacy. At room temperature, at 30 days, ceftazidime had a 26% decrease in efficacy and at 37°C at 30 days, there was no bioefficacy (100% decrease).

Amikacin was stable for all time points at the following temperatures: -80°C and -20°C. It was stable for 90 days at 6°C and at room temperature, with a 12% decrease in bioefficacy at 180 days at both of these storage temperatures. At 37°C, amikacin had a 10% or greater decrease in bioefficacy at all the time points. Amikacin showed an increase in efficacy at 90 days at -80°C (20% increase) and at 180 days at -20°C (18.7% increase).

Figure 1 shows the percent change in bioefficacy of all three antibiotics at all the time points and storage temperatures and the bold black line represents the 10 percent line.

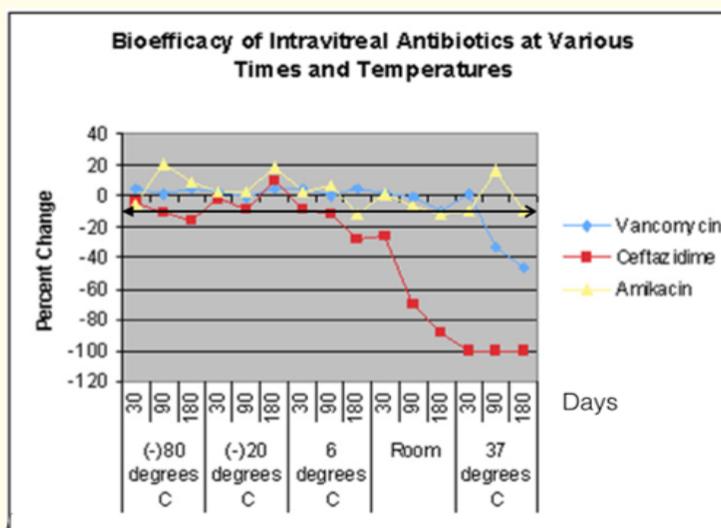


Figure 1: This graph shows the percent change in bioefficacy from time zero for each antibiotic (vancomycin = blue, Ceftazidime = red, amikacin = yellow) at specific time points and at specific temperatures.

Discussion and Conclusion

A delay in treatment for endophthalmitis post-intraocular surgery or post-intravitreal injections can have major implications. The purpose of this study was to determine if storage of antibiotics frequently used for endophthalmitis would result in reduced *in vitro* efficacy thus providing potential ineffective treatment.

Based on our results, we found that the two antibiotics most commonly used in endophthalmitis treatment, vancomycin and ceftazidime, are bioefficacious at various time points and temperatures. Vancomycin retains its efficacy for 180 days at -80°C, -20°C and 6°C. We found that ceftazidime retains its efficacy for 30 days at -80°C, -20°C and 6°C. For patients that are beta-lactam sensitive, amikacin can be used and we found that it retains efficacy for 180 days at -80°C and -20°C or for 90 days at 6°C. In this experiment, the increase in efficacy from baseline for amikacin suggests that the drug is more potent with time, which is unclear and may actually be a result of experimental error. A 6°C refrigerator is commonly found at most practice settings so vancomycin can be bioefficacious for 180 days, ceftazidime for 30 days and amikacin for 90 days.

Our results are different in storage times than what has been previously documented in the medical literature. Mehta, *et al.* [4] described a zone of inhibition technique in measuring potency of vancomycin and ceftazidime and found that the antibiotics were stable for 24 weeks at -20 and -80°C. Dobrinan, *et al.* [5] also showed similar results with stability of both vancomycin and ceftazidime at -18°C for 6 months. Similarly, this was the case for vancomycin in our study but not for ceftazidime when looking at bioefficacy. The issue with these studies is that a zone of inhibition method does not allow for precise measurement under a millimeter, thus exact measurements are difficult to deduce.

Of note, our study was not done using frost-free freezers, which are more commercially available. In a frost-free freezer, the temperature fluctuates to keep frost from building on to the coils that cool the freezer. Our freezers are kept at a constant temperature. It is possible that the results of this study could be different if repeated in a frost-free freezer.

In addition, the implications of this study are important because currently, in ophthalmology, many of our treatment options, especially intravitreal injectables are not FDA regulated or approved and are off-label uses. This is also the case with intravitreal preparations of antibiotics such as the ones that were studied in this experiment. Given this information, it becomes even more crucial for ophthalmologists to be prudent about the medications they receive from commercial compounding pharmacies as there are no FDA approved guidelines for preparation of these medications. Inpatient compounding pharmacies, on the other hand, do have national guidelines for sterile drug preparation that they must strictly adhere to. However, not all ophthalmic practices have access to an inpatient compounding pharmacy. This study may guide clinical practitioners in obtaining these antibiotics from pharmacies that they trust and rely in, and may be able to store these antibiotics for prompt treatment instead of sending patients to academic hospitals where inpatient compounding pharmacies are available.

Currently, in our clinical academic setting, we store these antibiotics for two weeks at -20°C. If the antibiotic is not used, it is then discarded. Based on the results of our study, these antibiotics have the potential to be stored for longer. In conclusion, we show that vancomycin 1 mg/0.1 mL can be stored for 6 months at 6°C or colder; ceftazidime 2.25 mg/0.1 mL can be stored for 30 days at 6°C or colder; and amikacin 0.4 mg/0.1 mL can be stored for 6 months at -20°C or colder without loss of bioefficacy in this *in vitro* model. These results may implicate a place for longer-term storage of intravitreal antibiotics used for the treatment of vision-threatening diseases, like endophthalmitis.

Acknowledgements

We are grateful to the Pennsylvania Lions Club, The Charles T. Campbell Foundation, Eye and Ear Foundation of Pittsburgh, PA, National Institutes of Health Core Grant P30 EY008098, and Unrestricted Grant from Research to Prevent Blindness, New York, NY, for continued financial support.

Conflict of Interest

No competing financial interests exist. The authors have no "Conflict of Interests" to disclose for the completion of this study as determined by the Office of Research, University of Pittsburgh, Pittsburgh, PA.

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Volume 9 Issue 11 November 2018

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