Rate of microbiological contamination of multi-use vials of bevacizumab and risk of endopthalmitis

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Abstract

Introduction: To determine the rate of microbiological contamination in multi-use vials of bevacizumab used for multiple intravitreal injections and to determine the rate of endophthalmitis in patients who received intravitreal injections from these vials.

Methods: Eighteen 100-mg (4 mL) vials of bevacizumab (*Avastin; F. Hoffmann-La Roche Ltd. Switzerland*) were used for 327 intravitreal injections in period of 18 months. These vials were stored in refrigerator at 2 – 8ºC for 30 days. From each vial, two samples of 0.05ml of avastin was withdrawn with the help of 1ml syringe at 7 and 30 days after the opening the vial. These samples were send for bacterial and fungal staining and culture. 327 patients who received intravitreal injections were monitored for signs and symptoms of inflammation or endopthalmitis.

Results: No evidence of microbiological contamination was detected in 18 vials sent for microbiological staining and culture. 327 patients who received intravitreal injections from these vials did not showed any signs or symptoms of inflammation or endopthalmitis.

Conclusions: Using the muti-use vials of bevacizumab for intravitreal injections which are stored for up to 30 days in refrigerator at 2–8ºC is safe.

Keywords: Bevacizumab, Endopthalmitis, Microbiological contamination, Multi-use vial.

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Introduction

Vascular endothelial growth factor (VEGF) inhibitors are used commonly for the treatment of wet age-related macular degeneration (ARMD), as well as for other inflammatory and neovascular retinal diseases, including retinal vein occlusion (RVO) and diabetic macular edema (DME). Studies have shown the efficacy and safety of these drugs in treatment of wet ARMD[1], RVO[2] and DME.[3] Intravitreal Anti-VEGF agents which are commonly used are bevacizumab (Avastin), ranibizumab (Lucentis), pegaptanib (Macugen) and aflibercept (Eylea). These agents are injected directly into the vitreous cavity, and are generally well-tolerated with a low overall complication rate.[1,2,3]

Bevacizumab *(Avastin; F. Hoffmann-La Roche Ltd. Switzerland)* is a full-length humanized VEGF inhibitor commonly used off-label in the treatment of AMD, RVO, and DME. Bevacizumab is cheaper than both ranibizumab and aflibercept. One recent study demonstrated that both aflibercept and ranibizumab are not cost-effective relative to bevacizumab unless their prices decrease by 69% and 80%, respectively.[4] CATT trial had demonstrated the equivalent effects of bevacizumab and ranibizumab on visual acuity in patients of neovascular ARMD.[1] Bevacizumab *(Avastin; F. Hoffmann-La Roche Ltd. Switzerland)* is dispensed as multi-use vials, which are stored in refrigerator at 2 – 8ºC. For intravitreal injections, 0.05ml of avastin is withdrawn with the help of 1ml syringe and injected intravitreally with 30G needle under sterile conditions.

Endophthalmitis is one of the most feared complication and has previously been reported to have an incidence after intravitreal injection of 0.02% to 0.05%.[1,5,6] The majority of causative organisms implicated in post intravitreal injection endophthalmitis were found to be Gram positive and Coagulase-negative Staphylococcus had been reported as most common organism.[5,7,8] Streptococcus species were found to be significantly more frequent after intravitreal injection than after intraocular surgery.[5] Intraocular inflammation was reported in 15 patients in Gujarat in December 2015 after intravitreal injection of Avastin which was later found to be associated with use of counterfeit bevacizumab.[9] One study had reported endophthalmitis in 12 patients after intravitreal injection of a contaminated group of pre-filled bevacizumab syringes prepared by the same compounding pharmacy. Culture results were positive for Streptococcus mitis/oralis in ten vitreous specimens.[10]

Concerns were made regarding dispensing of avastin in multi-use vials. This study had evaluated the microbiological contamination of 0.05ml of avastin

after being withdrawn from multi-use vials by 1ml syringe after 7 days and 30 days of opening the vials, which were stored in refrigerator at 2 – 8ºC. This study is similar to protocol followed by us in our clinical practise of storing the vial in refrigerator at 2 – 8ºC and using upto 30 days after opening the vial. Patients who received intravitreal injections were also monitored for development of signs and symptoms of inflammation or endopthalmitis.

Materials and Methods

Approval for this study was obtained from the institutional review board. All research and data collection adhered to the tenets of the declaration of helsinki and good clinical practice guidelines. 18 vials of bevacizumab *(Avastin; F. Hoffmann-La Roche Ltd. Switzerland)* were used in period of 18 months. After opening, the vials were stored in refrigerator at 2 – 8ºC. From each vial, two samples of 0.05ml of avastin was withdrawn with the help of 1ml syringe at 7 and 30 days after the opening the vial. These samples were send to laboratory for bacterial and fungal microbiological staining and culture. In the microbiological laboratory, Gram and KOH staining was done from AVastin sample for identifying bacterial and fungal pathogens respectively. Avastin sample was then inoculated on blood agar and MacConkey agar for bacterial culture, on Robertson's cooked meat medium for anaerobic culture and on Sabouraud Dextrose Agar for fungal culture.

327 patients received intravitreal injections from these 18 vials in 18 months. All patients were given a detailed explanation of the procedure and its potential risks and benefits and the off-label status of bevacizumab. Written consent was obtained from every patient. The treatment was given in the operating theatre under sterile conditions. Before injection, the conjunctiva bulbi, fornices, eyelid margins and lashes were rinsed repeatedly with povidone-iodine and topical anesthesia was induced by applying 0.5% proparacaine at least three times at 5-10 minutes intervals. 0.05ml of avastin is withdrawn with the help of 1ml syringe and injected intravitreally with 30G needle through the pars plana at a distance of 3.5-4.0 mm from the limbus. The needle was removed carefully using a sterile cotton applicator to prevent reflux. Indirect ophthalmoscopy was used to confirm uneventful intravitreal placement of the drug and perfusion status in central retinal artery. These patients were examined regularly at follow-up visits after 1 day, 7 days and 30 days and any signs and symptoms of inflammation or endopthalmitis such as pain, decreased vision, floaters, conjunctival congestion, eyelid edema, corneal edema, hypopyon, vitritis, scattered retinal hemorrhages and periphlebitis were closely monitired.

Results

0.05ml of avastin is withdrawn with the help of 1ml syringe from after 7 and 30 days of opening the vial from 18 vials of bevacizumab *(Avastin; F. Hoffmann-La Roche Ltd. Switzerland)* and send for bacterial and fungal microbiological staining and culture. No bacterial and fungal pathogens were identified on Gram and KOH staining respectively from 7 day sample and 30 day sample from 18 vials. No growth was documented from blood agar, MacConkey agar, Robertson's cooked meat medium and Sabouraud Dextrose Agar inoculated from 7 day sample and 30 day sample from 18 vials.

327 patients received intravitreal injections from these 18 vials in 18 months. 128 patients received injections for CNVM, 112 patients received injections for diabetic retinopathy and 87 patients received injections for retinal venous occlusions. 179 patients were males and 148 patients were females. These patients were examined regularly at follow-up visits after 1 day, 7 days and 30 days and any signs and symptoms of inflammation or endopthalmitis such as pain, decreased vision, floaters, conjunctival congestion, eyelid edema, corneal edema, hypopyon, vitritis, scattered retinal hemorrhages and periphlebitis were closely monitired. None of 327 patients had shown any signs and symptoms of inflammation or endophthalmitis during the follow-up period. No other severe ocular or systemic adverse events (such as retinal detachment, vitreous haemorrhage, endophthalmitis, cataract, hypertension, heart failure, or thromboembolic event) were noted.

Discussion

Vascular endothelial growth factor (VEGF) inhibitors have shown to be efficacious and safe in treatment of wet ARMD[1], RVO[2] and DME.[3] Bevacizumab is cheaper than ranibizumab, pegaptanib and aflibercept.[4] CATT trial had demonstrated the similar efficacy of bevacizumab and ranibizumab.[1]

There is lot of concern about safety of avastin injection in multi-use vials especially after incidence of intraocular inflammation in 15 patients in Gujarat in December 2015. It was later found to be associated with use of counterfeit bevacizumab.[9] Similarly, twelve eyes of 12 patients developed endophthalmitis after receiving intravitreal bevacizumab prepared by a single compounding pharmacy in south Florida in year 2011. An FDA investigation of the compounding pharmacy noted deviations from standard sterile technique, inconsistent documentation, and inadequate testing of equipment required for safe preparation of medications.[10,11] To ensure authenticity of the avastin vial and to address the challenge of counterfeit avastin, avastin vials should be purchased from a ROCHE authorized distributor and Kezzler code should be used. This is an alphanumeric code that is printed on the carton of avastin. This code needs to be sent as sms to the number indicated on the vial. The company

(ROCHE) will instantaneous reply about the authenticity of the vial. The vial should only be used after authenticating it.

Our study had send samples from 18 vials of bevacizumab in period of 18 months for bacterial, fungal and anaerobic microbiological culture. The samples were taken in a similar way in which injections are prepared for intravitreal injections in clinical practise. The samples were taken 7 days and 30 days after opening the vial. This is because of our current practise of using vials upto 30 days after opening them. The vials were stored in refrigerator at 2 – 8ºC similar to the trend followed in our current clinical practise. We found that no bacterial and fungal pathogens on Gram and KOH staining respectively from 7 day sample and 30 day sample from 18 vials. No growth was documented from blood agar, MacConkey agar, Robertson's cooked meat medium and Sabouraud Dextrose Agar inoculated from 7 day sample and 30 day Avastin sample from 18 vials. Similarly, Smith et al. (2008) had demonstrated no evidence of microbiological contamination in 21 vials sent for assessment.[12]

327 patients who had received the intravitreal injections from these 18 vials had not shown any sign of inflammation or endophthalmitis during the follow-up period. No other severe ocular or systemic adverse events (such as retinal detachment, vitreous haemorrhage, endophthalmitis, cataract, hypertension, heart failure, or thromboembolic event) were noted in these patients.

Based on the results of our study we conclude that current method of dispensing bevacizumab in multi-use vials and storage of these vials in refrigerator at 2 – 8ºC and upto 30 days is a safe method. Using the same vial multiple times for intravitreal injections do not increase the chances of endophthalmitis. Other studies had also shown that reasons behind cluster cases of endophthalmitis after avastin injection were either due to use of counterfeit bevacizumab or faults in preparation of injections and not due to dispensing bevacizumab in multi-use vials.

There were two major limitations of our study. Firstly, we have not send samples at 1st day of opening the vial because this study was primarily designed to evaluate safety of using multi-dose vials of avastin and storing in refrigerator at 2 – 8ºC for 30 days thus 1st day sample for assessing the sterility of new avastin vial was not included in protocol. Secondly, we had used bacterial and fungal microbiological staining and culture and not more sensitive tests of detecting microbiological contamination like polymerase chain reaction (PCR). In future, larger studies using more sensitive methods of detecting microbiological contamination like PCR are required to determine the safety and efficacy of this practice.

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