

Assessment of Effective Dose Preparability of Bevacizumab for Intraocular Administration

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ABSTRACT

Purpose: To evaluate the effective dosing of Bevacizumab in the operating room conditions divided by the form suitable for parenteral use and identify individual differences in its preparation.

Materials and Methods: Intravitreal Bevacizumab was prepared in 3 different groups from 100 mg / 4 ml Bevacizumab (Altuzan-Roche / USA) vial produced for parenteral use under laboratory conditions. The first two groups were formed by two blinded ophthalmologists, who had previously had experience in preparing intravitreal injections, by asking them to prepare 12 intravitreal Bevacizumab doses (1.25 mg / 50 µl) of insulin injectors. Group 3 was prepared by taking a fixed dose of 50 µl volume using a Hamilton injector. Samples were evaluated by spectrophotometric analysis. The calibration graph was drawn and the results obtained in the study were determined within the 95% confidence interval.

Results: Samples were evaluated in three groups. The mean amount of Bevacizumab prepared in Group 1 was 84.964 ± 6.03 µl, while it was 58.48 ± 5.65 µl in Group 2. The difference was statistically significant between the two groups. There was also a statistically significant difference between the two groups with the samples prepared using a Hamilton injector.

Conclusion: The use of intravitreal bevacizumab is discussed with the latest decision for intravitreal injections, especially because of the increased risk of endophthalmitis. In addition, it is not possible to prepare a fixed amount of medicine for each patient due to handmade division. The drug should be available in a disposable syringe suitable for intravitreal use.

Key Words: Bevacizumab, Intravitreal Injections, spectrophotometry, Clinical effectiveness.

INTRODUCTION

Bevacizumab (BVZ) is a mouse-derived monoclonal Ig G antibody that inhibits all isoforms of human vascular endothelial growth factor -A (VEGF-A).¹ Although it is currently an approved drug for the intravenous treatment of colorectal cancers, it has been used as an indication for retinal and corneal diseases that have been progressing with neovascularization for approximately 15 years.^{2,3} Since it is not approved for intravitreal use, there is no single-use preparation that has been prepared in a dose suitable for intraocular use.

In the United States of America (USA) and Europe, BVZ is prepared and repackaged for patients in a sterile syringe as a single-use preparation at a dose of 1.25 milligrams (mg)/50 microliters (µl) and it is applied to patients in

the operating room like aflibercept or ranibizumab.⁴ The absence of such pharmacy unit in our country has made it necessary to prepare BVZ in a dosage of 1.25 mg/50 µl by a healthcare professional in the operating room conditions, from which is suitable for parenteral use.

With the Health Practice Notifications, BVZ became compulsory to be used as a first-line treatment in diabetic macular edema (DME), macular edema due to retinal vein occlusions and age-related macular degeneration (AMD). While this situation increases the frequency of use of the drug, it carries some risks during its preparation and administration. Preparation and application in operating room and sterile conditions do not prevent the increased risk of endophthalmitis compared to other intravitreal preparations.⁵ Although the risk of endophthalmitis is the most emphasized and discussed topic, application

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and dosing standardization is the other important factor affecting the effectiveness of the treatment.

Our aim in this study is to evaluate the efficacy of BVZ, which is divided and applied from the form suitable for parenteral use in the operating room conditions, and to reveal personal differences during the preparation phase.

MATERIAL AND METHODS

The study was carried out in the Pharmaceuticals, Medical Devices and Dermocosmetic Laboratory in the Innovative Technologies Research Center of the university. Ethics committee approval was received from local Clinical Research Ethical Committee.

Intravitreal BVZ was prepared in 3 different groups from the vial of 100 mg/4 ml (Altuzan- Roche/USA) produced for parenteral use under laboratory conditions. The first two groups were made up by two blind ophthalmologists, who had previous experience in preparing intravitreal injection, by preparing 12 intravitreal BVZ doses (1.25 mg/50 μ l). The third group was prepared by using a Hamilton injector (Hamilton 800 series glass injector, -805 RN pst3-Hamilton Company, USA) with a fixed dose of 50 μ l.

In the second stage, 10 doses of BVZ were taken with a fixed volume of 30, 40, 50, 60 and 70 μ l using a Hamilton injector and evaluated by spectrophotometric analysis. In order to determine the amount of BVZ active substance in the sample prepared, the absorption-concentration graph was determined by comparing the calibration graph drawn with the measurement spectrum values of the samples. This graphic was used to determine the drug concentration in the dose of BVZ prepared in the groups.

The analysis portions prepared from the samples were analyzed with a double light path spectrophotometer at a wavelength of 190-1100 nm at 10 nm intervals and the appropriate wavelength range was determined. Then, measurements were made in the appropriate range of 190-280 nm wavelength. Ultra violet and visible region (UV/Vis) spectrum measurements were performed at 25 °C using computer controlled 1 cm bus length quartz cells and Lambda 35 Perkin-Elmer UV-Vis spectrophotometer device (Waltham, Massachusetts, U.S.A.). A calibration graph was drawn for the BVZ active ingredient and it was determined that the analytical method used was within the 95% confidence interval and performed measurements with the repeatability results obtained before the analyzes performed. The obtained results were evaluated by using data analysis with chemometric method.

RESULTS

Firstly, in order to determine the amount of BVZ active substance, the absorption-concentration graph was drawn by comparing the measurement spectrum values of the samples prepared with the Hamilton injector and the calibration graph drawn. The samples taken were then evaluated in three groups. While the average amount of BVZ prepared in the first group was 84.96 ± 6.03 μ l, this amount was measured as 58.48 ± 5.65 μ l in the second group ($p < 0.001$). There was also a statistically significant difference between 1.25 mg/50 μ l samples prepared using the Hamilton injector of the two groups ($p < 0.001$) (Figure 1 and 2).

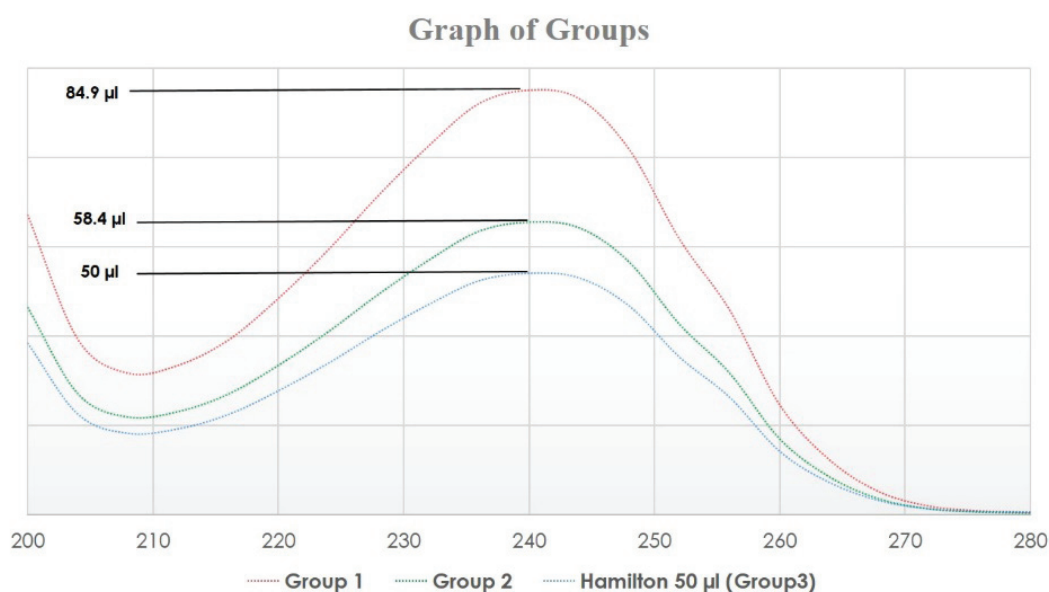


Figure 1. Graphic representation of the groups.

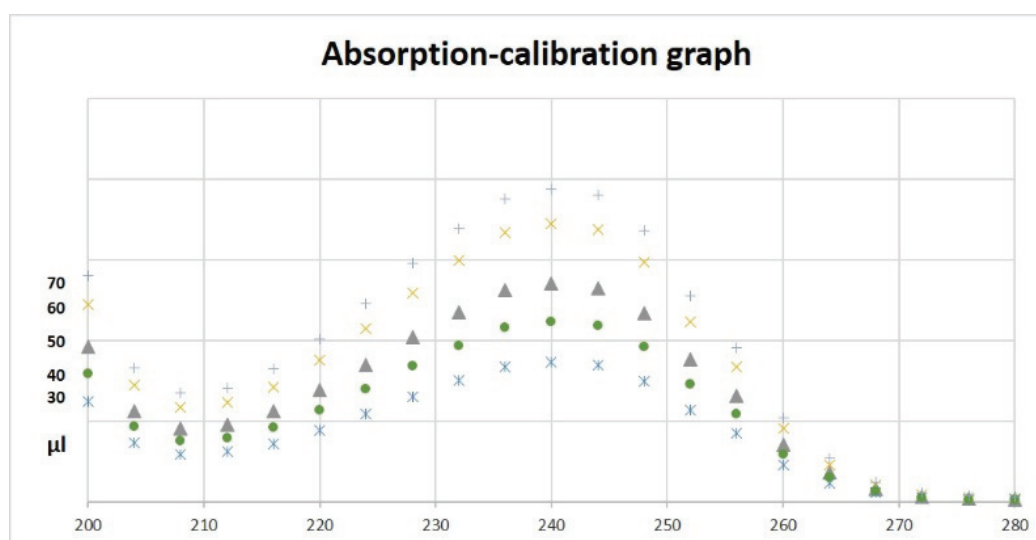


Figure 2. Absorption-concentration graph. (X-axis: nanometers, Y-Axis: microliters).

DISCUSSION

In our study, two different ophthalmologists who have previously prepared and administered intravitreal injection were asked to prepare intravitreal BVZ, and the samples were compared with reference samples prepared with Hamilton injector. A statistically significant difference was found between the samples prepared and both with the reference samples. Groups show consistency within themselves. This study reveals that every intravitreal injection prepared is different, therefore it is necessary to discuss this issue with regard to drug efficacy.

In the USA and Europe, intravitreal BVZ is presented to the user as pre-filled and sterilized syringes by compounding pharmacy units. The efficacy and safety of the drug in these syringes have been evaluated in many studies. Yannuzzi et al compared the final products of 11 different pharmaceutical companies in the USA; all the products were negative in terms of microbial or endotoxin contamination, but protein concentrations were not standardized despite strict laws declared by Food and Drug Administration.⁶ Similarly, another study comparing 5 different products in the UK revealed that there were differences in product quality and particle density.⁷ An Enzyme Linked Immunosorbent Assay (ELISA) study designed to analyze repackaged bevacizumab from 3 different compounding pharmacies in the United States revealed that 2 of the compounding pharmacies' batches had significantly less functional IgG in solution.⁸ Even one milliliter tuberculin syringe use reduces the effectiveness of intravitreal agents.⁹ Lowenstein et al evaluated the effectiveness of intravitreal drug administration in clinical practice and demonstrated the variability in the given volume by measuring the volume discharged by three different Anti-VEGF agents presented

ready for administration in a single dose injector.¹⁰ Even the standardization of products prepared by repackaging units specialized in this field and equipped with technological equipment is controversial. In our country, this type of institutional structure does not exist and intravitreal BVZ is dosed by healthcare professional before application. Therefore, dose and efficacy standardization of the drug is not possible.

Ultraviolet (UV) absorbance measurements such as UV/Vis double beam spectrophotometer provide a rapid and reliable method to determine protein concentrations.¹¹ With double beam system; the absorption of a buffered cuvette placed in the reference beam is subtracted from the absorbance measured for the sample to improve reliability. The prominent point for us in the study was whether there was a difference between the groups. Exact BVZ amount of each sample could be determined with ELISA as described before.⁶ The small number of samples and fewer groups can be considered as the limitations of the study, also.

With the final decision for intravitreal injections, the use of intravitreal BVZ is controversial, especially due to the increased risk of endophthalmitis. However, since it is prepared by division, it is difficult and troublesome to prepare medicines in an effective dose and amount for each patient individually. Repackaged single use forms of the drug should be available in a disposable syringe suitable for intravitreal use.

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