Assessment of bevacizumab quality and stability in repackaged syringes for clinical use

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ABSTRACT

Objectives Among measures taken to optimise financial resources, the off-label use of bevacizumab (Avastin) in the treatment of age-related macular degeneration (AMD) involves its repackaging from higher volume dosage forms. This use requires studies to analyse the viability of the repackaged preparations to ensure their quality, safety and efficacy. Our aim was to assess the structural stability and particle size of bevacizumab after it was repackaged from the original glass vials and stored in plastic syringes.

Methods High performance liquid chromatography by size exclusion (HPLC-SE) was used to quantify the bevacizumab and determine its degradation products after stress stability testing, with a particle size counter employed after repackaging and subsequent storage.

Results The syringes stored for 3 days at 4°C maintained the area of the main chromatographic peak above 100±10% of its initial value, and the observed particle size is the same as at baseline (20 nm) but with a double distribution towards larger sizes.

Conclusions This study shows how the repackaging of Avastin in plastic syringes permits their use for 3 days if stored under normal refrigeration. In this way, hospital pharmacy services can help optimise health resources without compromising the pharmaceutical standards of the drug.

INTRODUCTION

Age-related macular degeneration (AMD) and diabetic retinopathy (DR) lead to degeneration of the cornea, causing significant vision deterioration that greatly affects patients' quality of life.¹ Moreover, these conditions have a high incidence worldwide and are leading causes of vision loss and blindness.² Although in recent years new drugs based on inhibition of angiogenesis have been introduced, treatment remains complex and controversial.³ Currently, three closely related drugs whose mechanism of action is based on blocking vascular endothelial growth factor-A (anti-VEGF-A) are used: bevacizumab (BEV), ranibizumab and aflibercept. Only the last two have been approved by the European Medicines Agency (EMA) for the treatment of AMD. Before the adoption of ranibizumab by the US Food and Drug Administration (FDA) in 2006, 'off-label' use of BEV for these conditions was common among ophthalmologists, and supported by several studies.^{4–8} Different trials have shown similar efficacy and safety between BEV and ranibizumab,^{9–10} including a large trial in 2011 which confirmed equivalent efficacy.¹¹ In 2012, two double-blinded prospective trials found the efficacy of intravitreal aflibercept and ranibizumab

to be similar over a 5-year period.^{12–14} Aflibercept is a more cost-effective AMD treatment than ranibizumab but not when compared to BEV.^{15–16} BEV is frequently repackaged¹⁷ from large, glass vials into multiple, smaller, single-use, plastic, prefilled syringes, thus increasing the cost-effectiveness of this drug for AMD treatment. However, there are increasingly frequent reports describing adverse events associated with intravitreal BEV: sterile intraocular inflammation,^{18–21} infectious endophthalmitis^{22–23} and elevated intraocular pressure.^{24–26} The reasons for these adverse events are unclear but may be related to the intrinsic properties of the drug and its method of manufacture,²⁷ although they have also been attributed to degradation of BEV as a result of repackaging in plastic syringes.^{28–30} Particulate matter might be released from the syringes, causing irritation and inflammation of the cornea.^{31–32} Additionally, the new packaging conditions may affect the stability of BEV as a function of storage time³³ and thereby compromise its effectiveness.²⁹ Signorello *et al*³⁴ have recently determined that storage of repackaged syringes at 37°C under UV radiation, even for short periods of time, leads to BEV degradation.

The main objective of this study was to evaluate the stability of BEV repackaged from the original glass vials into plastic syringes, by analysing possible changes in the structure of the drug or baseline characteristics of the formulations caused by the repackaging process and subsequent storage under different stress conditions. High performance liquid chromatography by size exclusion (HPLC-SE) was used to characterise the underlying structural changes of the active molecule and the particle size found in repackaged formulations compared to the original vials.

METHODS

BEV was obtained from vials of the commercially available product Avastin.

Stability study

BEV was repackaged in 1 mL syringes (BD Plastipak, Spain) in laminar flow according to good manufacturing practices. Syringes were loaded with 200 µL for HPLC-SE determination, and with 900 µL for particle size assays, from two original vials of commercial Avastin (25 mg/mL) and stored at 4°C. Two syringes were loaded from each vial and analysed each at 0, 3, 7 and 15 days. Other stability tests were also performed in order to study BEV degradation behaviour: 30 µg/mL samples of BEV solution prepared from vials were subjected to different shear rates using Vortex (Atomixer Biotron, Spain) and Ultra-Turrax T-25 (Janke

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Kunkel Ika Labortechnik, Spain) instruments, and to different temperatures (37°C and 40°C).

HPLC-SE method

An HPLC-SE system (Waters, Milford, Massachusetts, USA) was used with Astra 6.0.1 data acquisition software (Chromatographic Manager, Waters). The stationary phase was a Shodex KW 804 (8.0 mm×300 mm) column and the mobile phase was phosphate-buffered saline (25 mM NaH₂PO₄·2 H₂O, 300 mM NaCl, pH 7) at a flow rate of 1.0 mL/min. UV detection was performed at 214 nm. All chemicals and reagents were HPLC grade. All solvents were filtered with 0.45 µm pore-size filters (Millipore, Billerica, Massachusetts, USA).

In order to validate the BEV analytical method,³⁵ five standard solutions were prepared at concentrations of 5–30.0 µg/mL. Each sample was analysed five times. Analysis of variance (ANOVA) of the linear regression confirmed the linearity of the method through rejection of the null hypothesis of linearity deviation for a significance level of 0.05 ($\alpha=0.05$); the coefficient of variation of the method was 3.9%. The equation of the regression line was: $\text{Area} = -28\,462 + 65\,201 \cdot C$; $r = 0.997$ ($n=25$), with a residual SE of 46 500. The method precision (as repeatability) was 0.01%, as determined by sixfold analysis of the same BEV sample. System accuracy was expressed as percentage recovery by the assay of a known added amount of drug, the mean value being $99.9 \pm 2.9\%$ ($n=9$). The detection and quantitation limits, based on the SD of the response and slope, were 2.3 and 7.0 µg/mL, respectively. A robustness test was performed to examine the effect of operational parameters on the analysis results. The flow rate (1.0 ± 0.08 mL/min), injection volume (50 ± 1 µL), temperature ($20.7 \pm 1.5^\circ\text{C}$) and column performance over time were determined in order to confirm the method's robustness. To calibrate the HPLC system and monitor its performance, a daily sample of BEV solution was analysed as standard. The estimated area for a standard concentration was 1 927 568 with an RSD of 3.0%. The upper and lower limits for the control chart were established at ± 3 SD of this value, taking as SD the value obtained from variance of the analytical method. Figure 1 shows the control chart for the method, where the peak's area holds between the established limits every time. The chromatographic conditions (flow rate, relative mobile-phase composition) and column performance, especially the tailing factor and column efficiency, were checked. When necessary, corrective action was taken.

The samples stored in syringes (200 µL) were suitably diluted with mobile phase for chromatographic analysis.

Particle size

Particle size distribution in samples stored in syringes (900 µL) was determined using a Zetasizer system (Malvern, UK).

RESULTS

Figure 2 shows the chromatogram of BEV obtained with the HPLC-SE method. Two peaks with an elution time of 8.6 and 9.8 min were detected. The first elution peak corresponds to a dimer species, while the second is the main monomer peak.³⁶

The evolution of the chromatographic peaks with the shearing force applied with the Vortex (figure 3A) and Ultra-Turrax (figure 3B) systems shows in both cases a reduction in the monomer area ($59.2 \pm 0.85\%$ and $95.6 \pm 0.12\%$, respectively) and the appearance of the trimer peak at approximately 6 min³² when more shear time is applied with the Ultra-Turrax instrument. Stability tests performed at 37°C and 40°C also show a reduction in the main peak (by $2.5 \pm 0.98\%$ and $2.7 \pm 2.96\%$,

Figure 1 High performance liquid chromatography by size exclusion (HPLC-SE) control chart for the bevacizumab (BEV) monomer area. The continuous line indicates the predicted value for a standard BEV concentration. The dashed lines indicate the established limits (± 3 SD). The filled circles indicate the monomer area value. A (µv.s) is the area of the chromatographic peaks.

respectively), but less than with the shear tests (figure 4). Variation in the BEV monomer levels corresponding to vials and repackaged syringes stored at 4°C is shown in figure 5. Only the BEV main peak area was studied. The area for the BEV monomer stored in Avastin vials remained between 90% and 110% of its initial value until day 7 of storage at 4°C. On the 15th day, this percentage had decreased to $71 \pm 0.9\%$. Analysis of variance established a statistically significant difference between days ($p < 0.05$) but not between vials ($p > 0.05$). For the BEV repackaged into syringes, only the initial area percentage was maintained at between 90% and 110% of baseline until day 3 of storage at 4°C. Analysis of variance of the remaining areas for BEV repackaged in syringes confirmed equal variances between them ($p > 0.05$).

Table 1 shows the particle sizes in Avastin vials and repackaged syringes stored at 4°C. For vials a single size distribution with approximately the same value (22 nm) at 0 and 15 days of storage at 4°C was found. Syringes also show the same starting

Figure 2 Bevacizumab chromatographic peaks (dimer at 8.6 min and monomer at 9.8 min).

Figure 3 Evolution of bevacizumab chromatographic peaks after different stirring times with Vortex (A), and ultra-Turrax (B) instruments. The circle in each figure shows the image enlarged.

and ending particle size, close to the value for the vials (19 nm). In the case of the syringes, from day 3 of storage a double size distribution was noted, towards higher particle sizes. Although the percentage by volume of this second size distribution sample is very small, it tends to progress towards larger sizes of about 5 μm at 15 days.

DISCUSSION

Health managers must sometimes optimise existing resources. One way to do this is to repackage drugs from higher to lower volume dosage forms for administration, but this requires studies to analyse the quality, safety and efficacy of the repackaged preparations. In the situation discussed here, although BEV is sold in 100 mg vials, intravitreal administration only requires a dose of 1.25 mg, such that plastic syringe repacking under laminar flow allows vials to be utilised fully for multiple administrations.

The proposed HPLC-SE method can detect the monomer and dimer of BEV, including their degradation products (trimer)

Figure 4 Evolution of bevacizumab chromatographic peaks after different storage times at (A) 37°C and (B) 40°C. The circle in each figure shows the image enlarged.

when the active substance is submitted to high shear forces. A decrease in the area of the main chromatographic peak (monomer) was detected for shear stress and temperature stability assays. As described by other authors,³⁶ aggregation of the molecule with shearing forces causes a decrease in the monomer area and the formation of high molecular weight transformation products, not detected by HPLC-SE as their molecular weight falls outside column limits.

Paul *et al*³⁰ demonstrated the stability of BEV repackaged in syringes and stored for 3 months at 4°C, showing that retention times and the relative percentages of the chromatographic peaks (monomer, dimer and high molecular weight species) do not change during this time. Unlike their study, we evaluated changes in the BEV monomer area with respect its initial value, and not the relative percentages between chromatographic peaks, as the total area, and not the relative percentages between peaks, may change. Our results show that BEV repackaged in syringes maintains the percentage of monomer area at $100 \pm 10\%$ of its initial value for only 3 days when stored at 4°C and that after this period, data variability increased with sampling time. However, for Avastin vials, the percentage is

Figure 5 Average percentage of bevacizumab monomer area remaining after storage of Avastin vials and repackaged syringes at 4°C.

maintained for up to 7 days. Other authors such as Liu *et al*²⁸ have detected some reduction in the amount of monomer present in the samples but have not explained the cause. Analysis of variance of these data found no statistically significant differences between the vials and repackaged syringes studied.

When particle size was analysed in the case of Avastin vials, the same particle size was detected throughout in the 15-day study with a single size distribution. In the case of repackaged syringes, the initial and final particle sizes were the same as in the vials, but from day 3 on, double size distribution increased towards far larger sizes with each further storage day. Other authors have detected a double size distribution at different storage conditions and even in control samples.^{30–34}

CONCLUSION

The HPLC-SE method was validated, allowing BEV to be detected and accurately and precisely quantified. Unlike previous studies carried out by other authors, this study shows how BEV repacked in plastic syringes from Avastin vials under laminar flow, maintains the initial percentage area of the main chromatographic peak (monomer) during 3 days of storage at 4°C. After checking the reduction in initial monomer area (time required for 10% degradation of the main chromatographic peak) and changes in particulate size or aggregation, we concluded that hospital pharmacy services can optimise health resources without compromising pharmaceutical standards.

Table 1 Particle size in vials and repackaged syringes after different storage times at 4°C

Vial 4°C			Syringe 4°C			
t (days)	Size (nm)	Volume (%)	t (days)	Size (nm)	Volume (%)	Size (nm)
0	22.3	100	0	19.0	100	—
3	12.6	100	3	17.2	100	252
7	15.0	100	7	30.0	99.8	4707
15	21.1	100	15	18.7	100	5299

Key messages

What is already known on this subject

- Elution times and the relative percentages between chromatographic peaks demonstrated that bevacizumab (BEV) repackaged in syringes was stable when stored at 4°C for 3 months.
- It was suggested that particulate might be released from BEV repackaged in plastic syringes.
- BEV degrades when stored in repackaged syringes at 37°C under UV radiation for short periods of time.

What this study adds

- High performance liquid chromatography by size exclusion was validated according to ICH guidelines.
- A reduction in the initial monomer chromatographic area and the evolution of particle size demonstrated the stability of bevacizumab obtained from Avastin and repackaged in plastic syringes and stored for 3 days at 4°C.

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Contributors FG, JN, JBF: conception and design; AS, ES-N: acquisition; AS, ES-N, JBF: analysis; AS, JBF: interpretation; AS, FG, JBF: drafted and critically revised the manuscript and agree to be accountable for all aspects of the work, ensuring integrity and accuracy; AS, JBF: gave final approval for publication.

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REFERENCES

- Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 1992;99:933–43.
- Romero-Aroca P. Managing diabetic macular edema: the leading cause of diabetes blindness. *World J Diabetes* 2011;2:98–104.
- Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 2004;25:581–611.
- Rich RM, Rosenfeld PJ, Puliafito CA, *et al*. Short-term safety and efficacy of intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Retina* 2006;26:495–511.
- Avery RL, Pieramici DJ, Rabena MD, *et al*. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmology* 2006;113:363–72.e5.
- Falkenstein IA, Cheng L, Morrison VL, *et al*. Standardized visual acuity results associated with primary versus secondary bevacizumab (Avastin) treatment for choroidal neovascularization in age-related macular degeneration. *Retina* 2007;27:701–6.
- Bashshur ZF, Schakal A, Hamam RN, *et al*. Intravitreal bevacizumab vs verteporfin photodynamic therapy for neovascular age-related macular degeneration. *Arch Ophthalmol* 2007;125:1357–61.
- Modarres M, Naseripour M, Falavarjani KG, *et al*. Intravitreal injection of 2.5 mg versus 1.25 mg bevacizumab (Avastin) for treatment of CNV associated with AMD. *Retina* 2009;29:319–24.
- Fong DS, Custis P, Howes J, *et al*. Intravitreal bevacizumab and ranibizumab for age-related macular degeneration: a multicenter, retrospective study. *Ophthalmology* 2010;117:298–302.
- De Rosa M, Messori A. The safety of bevacizumab and ranibizumab in clinical studies. *Int Ophthalmol* 2015;5:157–8.
- Martin DF, Maguire MG, Ying GS, *et al*. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011;364:1897–908.
- Heier JS, Brown DM, Chong V, *et al*. VIEW 1 and VIEW 2 Study Groups. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology* 2012;119:2537–48.
- Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, *et al*. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. *Ophthalmology* 2014;121:193–201.
- Lu G, Ades AE. Combination of direct and indirect evidence in mixed treatment comparisons. *Stat Med* 2004;23:3105–24.

- 15 Elshout M, van der Reis MI, Webers CA, *et al.* The cost-utility of aflibercept for the treatment of age-related macular degeneration compared to bevacizumab and ranibizumab and the influence of model parameters. *Graefes Arch Clin Ophthalmol* 2014;252:1911–20.
- 16 Shaikh AF, Toussaint BW, Miller DM, *et al.* Cost comparison of intravitreal aflibercept with bevacizumab and ranibizumab for the treatment of wet age-related macular degeneration. *Ophthalmic Surg Lasers Imaging Retina* 2015;46:62–6.
- 17 Wells JA, Glassman AR, Ayala AR, *et al.* Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med* 2015;372:1193–203.
- 18 Bakri SJ, Larson TA, Edwards AO. Intraocular inflammation following intravitreal injection of bevacizumab. *Graefes Arch Clin Exp Ophthalmol* 2008;246:779–81.
- 19 Yamashiro K, Tsujikawa A, Miyamoto K, *et al.* Sterile endophthalmitis after intravitreal injection of bevacizumab obtained from a single batch. *Retina* 2010;30:485–90.
- 20 Ness T, Feltgen N, Agostini H, *et al.* Toxic vitritis outbreak after intravitreal injection. *Retina* 2010;30:332–8.
- 21 Medicines and Healthcare Products Regulatory Agency 2012. Company-led drug recall: Bevacizumab Intravitreal Injection 1.25 mg/0.05ml - Moorfields Pharmaceuticals - CLDA(12)A/04. <http://trove.nla.gov.au/work/163636137?versionId=178402218> (accessed Dec 2015).
- 22 Artunay O, Yuzbasioglu E, Rasier R, *et al.* Incidence and management of acute endophthalmitis after intravitreal bevacizumab (Avastin) injection. *Eye* 2009;23:2187–93.
- 23 Goldberg RA, Flynn HW Jr, Isom RF, *et al.* An outbreak of streptococcus endophthalmitis after intravitreal injection of bevacizumab. *Am J Ophthalmol* 2012;153:204–8.
- 24 Good TJ, Kimura AE, Mandava N, *et al.* Sustained elevation of intraocular pressure after intravitreal injections of anti-VEGF agents. *Br J Ophthalmol* 2011;95:1111–14.
- 25 Adelman RA, Zheng Q, Mayer HR. Persistent ocular hypertension following intravitreal bevacizumab and ranibizumab injections. *J Ocul Pharmacol Ther* 2010;26:105–10.
- 26 Choi DY, Ortube MC, McCannel CA, *et al.* Sustained elevated intraocular pressures after intravitreal injection of bevacizumab, ranibizumab, and pegaptanib. *Retina* 2011;31:1028–35.
- 27 Palmer JM, Amoaku WM, Kamali F. Quality of bevacizumab compounded for intravitreal administration. *Eye* 2013;27:1090–7.
- 28 Liu L, Ammar DA, Ross LA, *et al.* Silicone oil microdroplets and protein aggregates in repackaged bevacizumab and ranibizumab: effects of long-term storage and product mishandling. *Invest Ophthalmol Vis Sci* 2011;52:1023–34.
- 29 Kahook MY, Liu L, Ruzyski P, *et al.* High-molecular-weight aggregates in repackaged bevacizumab. *Retina* 2010;30:887–92.
- 30 Paul M, Vieillard V, Roumi E, *et al.* Long-term stability of bevacizumab repackaged in 1 mL polypropylene syringes for intravitreal administration. *Ann Pharm Fr* 2012;101:21–30.
- 31 Weber AJ, Zelenak D. Experimental glaucoma in the primate induced by latex microspheres. *J Neurosci Methods* 2001;111:39–48.
- 32 Urcola JH, Hernández M, Vecino E. Three experimental glaucoma models in rats: comparison of the effects of intraocular pressure elevation on retinal ganglion cell size and death. *Exp Eye Res* 2006;83:429–37.
- 33 Bakri SJ, Snyder MR, Pulido JS, *et al.* Six-month stability of bevacizumab (Avastin) binding to vascular endothelial growth factor after withdrawal into a syringe and refrigeration or freezing. *Retina* 2006;26:519–22.
- 34 Signorello L, Pucciarelli S, Bonacucina G, *et al.* Quantification, microbial contamination, physico-chemical stability of repackaged bevacizumab stored under different conditions. *Curr Pharm Biotechnol* 2014;15:1–7.
- 35 ICH Q2(R1), 2014, in: The International Conference on Harmonisation. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf (accessed Mar 2016).
- 36 Oliva A, Llabrés M, Fariña JB. Capability measurement of size-exclusion chromatography with a light-scattering detection method in a stability study of bevacizumab using the process capability indices. *J Chromatogr A* 2014;353:89–98.