Extended Stability and Sterility of Antihemophilic Factor Human

Sarah Shields, PharmD; Abby Kim, PharmD; and Joshua Elder, PharmD

OBJECTIVES Antihemophilic factor human is a factor VIII product used to supplement those with hemophilia. Recent data show treatment benefit and cost saving opportunities if factor products are administered as a continuous infusion rather than conventional bolus dose. This method has not been widely used given the lack of evidence for safe and effective use beyond 3 hours from preparation. The objectives of this study were to determine the physical and chemical stability and sterility of antihemophilic factor human over a 7-day period.

METHODS Antihemophilic factor human was obtained from the manufacturer. Baseline stability and sterility were determined by factor activity levels along with bacterial and fungal cultures. These tests were also evaluated over a span of 7 days at room temperature and under refrigeration.

RESULTS Each sample was inspected at the time of delivery and showed no visible signs of physical changes. Factor activity levels were maintained between 88% and 102% of baseline measurements. No growth was observed for bacterial or fungal cultures in any sample after 4 weeks of incubation.

CONCLUSIONS Antihemophilic factor human maintained physical stability and chemical stability and remained sterile for the 7-day period, allowing extended stability and continuous infusions to be considered.

KEYWORDS antihemophilic factor; drug stability; factor VIII, hemophilia

J Pediatr Pharmacol Ther 2017;22(3):203–206

DOI: 10.583/1551-6776-22.3.203

Introduction

Hemophilia is an X-linked genetic bleeding disorder characterized by a deficiency in clotting proteins factors VIII and IX.¹ Hemophilia affects approximately 1 in 10,000 newborns.² Hemophilia A is caused by a lack of factor VIII and is 4 times more common than hemophilia B, which is caused by a lack of factor IX.¹ Hemophilia can cause severe and life-threatening consequences if left untreated, such as excessive bleeding from any injury, that could lead to death. The current first-line treatment option for these patients is replacement of the specific deficient factor. For treatment of hemophilia A, there are 2 current factor replacement categories, human and recombinant.¹ Concerns with human plasma-derived products include the risk of transmitting blood-borne diseases. Although advanced screening techniques have reduced this risk significantly, these products can still be subject to historical stigma and still maintain a slight risk.³ Human plasma-derived products are the original factor concentrates introduced to the market. Recombinant products were created after the factor VIII gene was cloned and replicated. There is also concern with the development of inhibitors; however, current reports do not identify one product that is more likely to lead to inhibitor development over another.¹

Antihemophilic factor human is a factor VIII product used as a therapeutic supplement for those with he-

mophilia A. Conventionally, this product is administered through bolus dosing, but recent data show treatment benefit and cost saving opportunities if a factor product is administered as a continuous infusion. A prospective controlled trial compared factor VIII bolus infusions with continuous infusions in patients with hemophilia undergoing a surgical procedure.⁴ The study showed a 36% reduction in total factor use for a 13-day treatment course when continuous infusion was used. A statistically significant increase in time above the desired factor VIII level was also observed as well as no significant bleeding events. A single-center, open-label, non-randomized phase III study evaluated not only the impact of continuous infusion but also the stability and sterility of recombinant factor VIII within the environment of the continuous infusion pump. All lots tested maintained greater than 80% activity for at least 48 hours as well as remained sterile. Continuous infusion maintained factor VIII plasma levels at or above goal for all patients, without producing any significant peaks or dangerous troughs.⁵ A similar study in a pediatric hospital showed that stable factor VIII levels can be maintained in pediatric patients.⁶ This product, which was further diluted in order to run in pediatric infusion pumps, nevertheless showed positive results for use as a continuous infusion. The main barriers for implementing this novel approach are concerns for stability and sterility of this product, as the manufacturer does

not report safe use beyond 3 hours from preparation.⁷

Previous studies have examined the stability and sterility of recombinant factor VIII concentrates as well as factor VII products. An *in vitro* analysis evaluated the stability and sterility of recombinant factor VIII at room temperature, under refrigeration, and above room temperature to simulate possible excursions. Through testing, it was found that all samples remained sterile and maintained greater than 80% factor activity levels for at least 18 months.⁸ Additional *in vitro* analysis evaluated recombinant factor VIII at room temperature and under refrigeration and found that the samples maintained full activity for 24 hours when refrigerated and for 6 hours at room temperature.⁹

Although data exist for recombinant factor products, no data are currently available that document prolonged stability and sterility for the specific human factor product. At a pediatric academic medical center, more than \$1.3 million was spent on antihemophilic factor human in just 1 year. Because this drug significantly impacts the pharmacy budget, alternative dosing strategies as well as having an extended-beyond-use date is desired. Policies have been enacted to prevent waste of these products, but no system is perfect, and product occasionally must be wasted.

The primary objective for this study was to determine the physical and chemical stability of antihemophilic factor human over a 7-day period at room temperature (up to 25°C) and under refrigeration (2°C to 8°C). The secondary objective was to assess sterility at room temperature and under refrigeration of the product over a 7-day period. This study provides the first analysis of antihemophilic factor human for stability and sterility beyond the recommended 3 hours due to the absence of preservatives and concern for microbial contamination.^{7,8}

Materials and Methods

This research was deemed exempt by the local Institutional Review Board. Five vials of antihemophilic factor human (Koate DVI, Grifols Therapeutics Inc., Research Triangle Park, NC) were donated from the manufacturer. Each vial was reconstituted with 10 mL of sterile water for injection, using aseptic technique, as defined by United States Pharmacopeia chapter 797.¹⁰ Thirty-nine 1-mL samples were extracted from the 5 vials and labeled. Each label contained the time the sample should be processed, the storage instructions, and the specific type of testing (i.e., activity level and bacterial and fungal cultures). Three samples were taken and immediately processed to determine baseline stability and sterility. One sample was used to determine Factor activity, one for bacterial contamination, and the third sample for fungal contamination. The remaining 36 samples were further divided evenly (12 samples in each testing group) for storage under refrigeration (5°C) and at room temperature (21°C). Samples were

hand delivered by the primary investigator to the institutional microbiology and hematology laboratories and tested at times 0, 1, 12, 24, 48, 72, and 168 hours. At the time of delivery, all samples were visually inspected for changes in clarity, color, and signs of particulate formation. Visual inspection was also completed by independent laboratory personnel.

Factor activity level was assessed for samples at both room temperature and under refrigeration at each time point, using a coagulometric assay performed using the BCS XP system (Siemens Healthcare Diagnostics, Inc.; Tarrytown, NY) using actin FSL reagent. When received by the laboratory, the sample underwent serial dilution to obtain the percent activity. The goal percent activity was set between 80% and 125% of the initial activity as required by the United States Food and Drug Administration.¹¹

Sterility was tested for samples at both room temperature and under refrigeration at each time point. To test for bacterial contamination, 1 mL of sample was inoculated into a 10-mL thioglycollate broth tube, which was incubated at 35°C for 14 days. To test for fungal contamination, 1 mL of sample was spread onto a plate of Sabouraud's dextrose agar and incubated under aerobic conditions at 30°C for 4 weeks. All culture results were determined by the institution's microbiology department.

Results

Antihemophilic factor human was evaluated for stability and sterility over the course of 7 days. The refrigerated and room temperature samples were delivered for factor activity evaluation as well as bacterial and fungal plating at hours 0, 1, 12, 24, 48, 72, and 168. Each sample was inspected visually upon delivery to the laboratory by the primary investigator, and all samples remained clear, colorless, and free of particulate matter, indicating physical stability. Initial factor activity was determined, and all samples maintained at least 90% activity over the course of 72 hours at both room temperature and under refrigeration. Furthermore, antihemophilic factor human maintained at least 88% activity over the 168-hour time period regardless of storage temperature, meeting the predetermined parameters of maintaining activity between 80% and 125% as seen in the Table. Reinforced graphically in the Figure, the percent change from baseline fluctuated within the desired range for the 7-day duration likely due to the allowable margin of error in the factor activity assay used. Antihemophilic factor human also maintained sterility, with no growth noted in either bacterial or fungal cultures after incubation for 4 weeks. This was determined to be the final reading of no growth by the microbiologist.

Discussion

Whether it was stored at room temperature or under

Table. Factor Activity Percent Change from Baseline

Time (hr)	Room Temperature (%)	Refrigeration (%)
0	100.00	100.00
1	100.19	100.00
12	94.39	101.72
24	93.09	91.04
48	98.56	101.35
72	94.62	93.64
168	88.77	88.72

refrigeration, antihemophilic factor human proved to be stable and sterile beyond the manufacturer's recommendation of 3 hours from reconstitution and for as long as 7 days. These results are consistent with the studies published evaluating the stability and sterility of recombinant factor VIII products as well as factor VII products. This allows clinicians the opportunity to consider continuous infusion to provide better patient outcomes as well as to decrease the amount of factor products consumed. Knowing that factor products are stable and sterile beyond 3 hours also provides the potential to avoid factor waste due to delayed administration. As previously mentioned, a comparison between conventional bolus dosing and continuous infusion showed that the continuous infusion used at least 36% less factor VIII concentrates than the bolus dosing.⁴ This extended stability data would allow our institution to convert to continuous infusion factor use, which could result in potential savings of \$468,000 for an institution that spends \$1.3 million annually on this specific product. In addition, antihemophilic factor human doses can regularly exceed \$5,000 per dose, especially in adolescent patients with high-titer inhibitors. Having extended stability data could prevent dose waste in certain patient care scenarios, such as patients losing intravenous access. This would translate into a significant cost savings to the institution, the healthcare system, and the patient.

A limitation of this study is seen by the variation in activity levels from hour 24 to 48. The activity level is expected to decrease, however an increase was observed. This may have been due to the sensitivity of the coagulometric assay and the serial dilution required to obtain a percent activity. Although this is considered a limitation, it is within the allowable range of variation for activity levels as defined by the United States Food and Drug Administration to be deemed bioequivalent.

An additional limitation of this study is that only 1 lot of antihemophilic factor human was used in testing. Evaluating different lots of this product would identify any variations within the manufactured product. This study evaluated the stability and sterility in vitro and does not account for any in vivo processes that may alter the stability in patients. With this study's goal to

Figure. Factor activity percent change from baseline

validate adequate factor activity levels beyond 3 hours, physical stability evaluation through visual observation is not sensitive enough to detect variations in pH or slight changes in color. Additional studies should be completed to evaluate physical stability in a more sensitive manner which will also limit potential observational bias.

Based on the results of this research, this institution has elected to extend the beyond-use date of antihemophilic factor human and will allow this product to be used up to 7 days after reconstitution. This novel research contributes to reports of the stability and sterility of plasma-derived factor products. To build on these results and provide a clinical benefit, additional studies should be carried out to address the use of antihemophilic factor human as a continuous infusion to support the studies previously completed on recombinant products. This study demonstrates physical and chemical stability of the product to allow for institutional extension of beyond use dating and consideration in continuous infusion treatment regimens.

ARTICLE INFORMATION

Affiliations Department of Pharmacy, Norton Children's Hospital (SS, AK, JE), Louisville, Kentucky

Correspondence Joshua Elder, PharmD, BCPS; Joshua.Elder@nortonhealthcare.org

Disclosure The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

Acknowledgment The product used in this research was donated by Kedrion Biopharma, Inc; however, the donor had no additional contribution or role in this study. All testing was

donated by the Norton Healthcare laboratory department. This research was presented as a poster presentation at the Pediatric Pharmacy Advocacy Group conference in Minneapolis, MN from April 27th – May 1st 2015.

Copyright Published by the Pediatric Pharmacy Advocacy Group. All rights reserved. For permissions, email: matthew.helms@ppag.org.

REFERENCES

1. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19(1):e1-e47.
2. Stonebraker JS, Bolton-Maggs PHB, Soucie JM, et al. A study of variations in the reported haemophilia: a prevalence around the world. *Haemophilia*. 2010;16(1):20-32.
3. American Society of Hematology. Hemophilia: from plasma to recombinant factors. Washington, DC: American Society of Hematology; Dec 2008 [cited May 13, 2015]. <http://www.hematology.org/About/History/50-Years/1524.aspx>. Accessed January 19, 2017.
4. Batorovo A, Martinowitz U. Intermittent injections vs. continuous infusion of factor VIII in haemophilia patients undergoing major surgery. *Br J Haematol*. 2000;110(3):715-720.
5. Martinowitz U, Luboshitz J, Bashari D, et al. Stability, efficacy, and safety of continuously infused sucrose-formulated recombinant factor VIII (rFVIII-FS) during surgery in patients with severe haemophilia *Haemophilia*. 2009;15(3):676-685.
6. Revel-Vilk S, Blanchette, VS, Schmugge M, et al. In vitro and in vivo stability of diluted recombinant factor VIII continuous infusion use in haemophilia A. *Haemophilia*. 2010;16(1):72-79.
7. Koate DVI [package insert]. Research Triangle Park, NC; Grifols Therapeutics Inc; 2012.
8. Parti R, Schoppman A, Lee H, et al. Stability of lyophilized and reconstituted plasma/albumin-free recombinant human factor VIII (ADVATE rAHF-PFM). *Haemophilia*. 2005;11(5):492-496.
9. Nedergaard H, Vestergaard S, Jensen PT, et al. In vitro stability of lyophilized and reconstituted recombinant activated factor VII formulated for storage at room temperature. *Clin Ther*. 2008;30(7):1309-1315.
10. Pharmaceutical compounding-sterile preparations (general information chapter 797). In: The United States pharmacopeia, 27th rev., and The national formulary, 22nd ed. Rockville, MD: The United States Pharmacopeial Convention, 2004:2350-70.
11. US Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry: statistical approaches to establishing bioequivalence. Rockville (MD): US Department of Health and Human Services, Food and Drug administration, Center for Drug Evaluation and Research; Jan 2001 [cited Jun 6, 2015]: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070244.pdf>. Accessed January 19, 2017.