CLINICAL INVESTIGATION

Stability and Sterility of Enoxaparin 8 mg/mL Subcutaneous Injectable Solution

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BACKGROUND: Enoxaparin is often diluted to accurately deliver doses to neonatal and infant patients. Current recommendations for dilutions may not be adequate for the smallest patients.

METHODS: Review of dosing at our institution occurred, and an 8 mg/mL concentration of enoxaparin was chosen. A concentration of 8 mg/mL was compounded by diluting 0.4 mL of enoxaparin (100 mg/mL) into 4.6 mL of sterile water for injection into an empty sterile vial. Four syringes of the 8 mg/mL concentration were prepared by 5 technicians (20 total syringes). Stability and sterility testing occurred at 0, 7, 14, and 30 days. One-way repeated-measures analysis of variance was used to detect significant differences in Anti-Factor Xa concentrations at the testing time points.

RESULTS: The dilution of enoxaparin was sterile at 30 days but exhibited significant degradation at the 30-day point ($p < 0.05$).

CONCLUSION: A dilution of enoxaparin 8 mg/mL is stable and sterile for 14 days refrigerated but is not stable at 30 days.

INDEX TERMS: Anti-Factor Xa concentrations, enoxaparin, low molecular weight heparin, neonates, stability, sterility

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BACKGROUND

The accurate delivery of medications to neonates and infants can be difficult.^{1–6} The youngest patients often require manipulation of commercially available products to accurately administer doses of medications. Examples of this include compounding of oral medications in liquid formulations and dilution of injectable formulations to provide adequate volumes for very small doses.⁷ Although use of enoxaparin is not approved for neonates by the Food and Drug Administration, off-label use of enoxaparin is not uncommon.^{1,8–11}

A dilution of enoxaparin has been published⁷ to help address this need in patients requiring anticoagulation. The published 20 mg/mL concentration is helpful to accurately deliver a dose of enoxaparin for patients who would otherwise have an unmeasurable volume of drug if the commercially available 100 mg/mL concentration was used. However, we have noticed in our

patient population that preterm and low-birth weight neonates may require a further dilution of enoxaparin to accurately deliver a prescribed dose. For example, a 1-kg patient with a dose of 1.7 mg (1.7 mg/kg/dose) would require a volume of 0.09 mL, which could be challenging to measure using a standard 1-mL syringe. A lower concentration dilution of enoxaparin would be useful in patient care scenarios with very small pediatric patients. There currently is no published information regarding a dilution of enoxaparin of less than 20 mg/mL.

The objective of this study was to determine if a low molecular weight heparin (LMWH) solution of 8 mg/mL maintains stability and sterility over a 30-day period.

METHODS

A review of dosing practices at our institution occurred for patients receiving the 20 mg/

mL concentration of enoxaparin. Based upon doses used and the titration requirements of the patients receiving the diluted enoxaparin, a concentration of 8 mg/mL was chosen.

To prepare the 8 mg/mL concentration, 5 syringes of 0.4 mL of enoxaparin (40 mg/0.4 mL) (Lovenox, Aventis, Bridgewater, NJ) were injected into 5 separate 10-mL sterile vials, and each was diluted with 4.6 mL of sterile water for injection. Each vial was prepared by a different certified pharmacy technician in an ISO Class 5 laminar flow hood in an ISO Class 7 room following USP 797 guidelines for preparation of compounded sterile products in a laminar flow hood. Each vial had 1 mL withdrawn into polypropylene syringes (Becton Dickinson Inc, Franklin Lakes, NJ), and the syringes were labeled with unique identifiers. In summary, 4 syringes were pulled from each of the 5 vials of enoxaparin, resulting in 20 syringes of 8 mg/mL concentration. Additionally, a 1-mL sample of normal saline in a syringe was used as a blank standard. The samples were stored under refrigeration at 2°C to 6°C during the study period. Laboratory personnel were blinded to the pharmacy technician who prepared the solution. The samples were all prepared on the same day and delivered to the laboratory on that day. Stability testing was performed by the same technician (GP) at each of the time points. Stability testing was conducted on the STAR Evolution (Diagnostica, Stago Inc, Parsippany, NJ) and was performed according to assay manufacturer specifications.

The samples underwent a 1:800 dilution prior to analysis with normal pooled plasma (NPP) (Precision Biologic Inc, Dartmouth, NH). This was done in a serial fashion starting with two 1:20 dilutions (25 μ L + 475 μ L NPP) and a final 1:2 dilution (250 μ L + 250 μ L NPP). This was then placed on the STAR using a LMWH protocol. The STAR liquid Anti-Factor Xa assay is intended for the quantitative determination of the plasma concentrations of LMWH by the measurement of their Anti-Factor Xa activity in a competitive system using a synthetic chromogenic substrate. The normal function of a molecule of Factor Xa, as soon as it appears in plasma, is to cleave its natural substrate, prothrombin, to generate thrombin, the enzyme responsible for the fibrin clot formation. In the presence of heparin, competition occurs between this mechanism and the inhibitory mechanism exerted by the heparin-antithrombin

complex, this inhibition being largely responsible for the action of heparin.

The proposed method is a 1-step reaction based on a similar principle: as soon as Factor Xa is added to the plasma-substrate mixture, 2 reactions take place simultaneously: hydrolysis of the substrate by Factor Xa and inhibition of Factor Xa by the heparin-antithrombin complex. After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of pNA (paranitroaniline) that is released is inversely proportional to the concentration of heparin present in the plasma. Low molecular weight heparins are used for the prevention and treatment of thromboembolic diseases. The quantitative determination of plasma LMWH concentration is a useful tool for monitoring treatment efficacy.

Sterility testing of the 5 aliquots and of the saline solution used for dilution was conducted on days 0, 7, 14, and 30. Sterility testing followed the USP Compounding Standards & Resources. Culture media was used; this included TSA w/5% blood, Chocolate, and Sabouraud Dextrose Agar plates. This combination of plates allows for the largest variety of bacterial and fungal growth needs. Each series of plates was inoculated on days 0, 7, 14, and 30 and observed for 14 days to detect any contaminate. Determination of microbial contamination was performed by hospital microbiology laboratory staff.

Results were presented in graphical format and as a table. A one-way repeated-measures analysis of variance (ANOVA) was used to determine significant differences in the concentrations at the various time points. Analysis was performed with Stata IC v.12 (StataCorp, College Station, TX).

RESULTS

Data for each syringe are presented in the Table. The mean concentration at day 0 was 8.49 ± 0.73 mg/mL, with a lowest mean concentration of 7.26 ± 0.71 mg/mL at day 30. Repeated-measures ANOVA demonstrated a significant difference in enoxaparin concentration at day 30 ($p < 0.001$). Graphical representation of enoxaparin concentration for each syringe (A-E) demonstrated decreased enoxaparin concentrations at day 30 but similar concentrations at day 7 and day 14 (Figure). There was no growth detected at 30 days for any of the syringes.

Table. Values for Anti-Factor Xa Stability Testing

Vial	% of Reference* Mean (Range)			
	Day 0	Day 7	Day 14	Day 30
A†	94% 7.52 mg/mL (6.843-8.197)	92% 7.36 mg/mL (6.698-8.022)	97% 7.76 mg/mL (7.062-8.458)	78% 6.24 mg/mL (5.678-6.802)
B†	115% 9.20 mg/mL (8.372-10.028)	103% 8.24 mg/mL (7.498-8.982)	115% 9.20 mg/mL (8.372-10.028)	92% 7.36 mg/mL (6.698-8.022)
C†	99% 7.92 mg/mL (7.207-8.633)	99% 7.92 mg/mL (7.207-8.633)	102% 8.16 mg/mL (7.426-8.894)	89% 7.12 mg/mL (6.479-7.761)
D†	112% 8.96 mg/mL (8.154-9.766)	109% 8.72 mg/mL (7.935-9.505)	112% 8.96 mg/mL (8.154-9.766)	92% 7.36 mg/mL (6.698-8.022)
E†	111% 8.88 mg/mL (8.081-9.679)	103% 8.24 mg/mL (7.498-8.982)	137% 10.96 mg/mL (9.974-11.946)	103% 8.24 mg/mL (7.498-8.982)

*8 mg/mL

†Four syringes were tested

DISCUSSION

This is the first report of an 8 mg/mL dilution of enoxaparin. The motivating factor for developing a more dilute concentration is the ability to accurately dose enoxaparin in very small infants.^{5,12} The limitations of current published dilutions are apparent in the titration of enoxaparin to achieve a goal Anti-Factor Xa concentration according to published guidelines.^{8-11,13-19} As a result of the difficulty in accurately measuring volumes, some centers have begun titrating of medications in whole-milligram increments, which is also our practice.²⁰ This can also be problematic with very small doses, as a patient receiving a 1-mg dose would experience a 100% increase in dose with a whole-milligram titration.^{3,21} We have also avoided using insulin syringes as a medication safety strategy.

As an example, if a 2-kg patient receives a starting dose of 3.4 mg/dose (1.7 mg/kg/dose) and subsequently requires a dose increase of 10% (0.34 mg), this change in volume, using a 20 mg/mL concentration, would be 0.017 mL. This volume would be too small to be accurately measured using currently available syringes. The 8 mg/mL solution would provide a larger volume (0.04 mL), which would make the delivery of doses in this patient population more accurate.

Overall, we would suggest using the 8 mg/mL concentration in patients receiving < 8 mg/dose

to ensure that potential subsequent titrations are accurate. Clinicians should also be aware of when to switch to the 20 mg/mL concentration so that as patients grow, they do not receive large subcutaneous injection volumes (>1 mL).

When stored in polypropylene syringes, enoxaparin was not stable over the 30-day period, as anticipated. Previous publications⁷ have noted that degradation does occur with the 20 mg/mL concentration but is not significant at 30 days.

The dilution we prepared had significant degradations at 30 days; therefore, we can only recommend using the dilution up to 14 days after preparation. While this is a limitation, as patients grow, they may be switched to the more concentrated preparation of enoxaparin, which has a longer stability. There was also significant variability in the concentrations (>10%), for which we were unable to identify a cause.

Future directions include the evaluation of the new concentration in our patient population. Our institution has adopted the practice of titrating enoxaparin to whole-milligram doses, with concern for safety in our smaller patients.²⁰ We plan on evaluating the use of the more dilute enoxaparin concentration in the smallest patient populations to determine if this concentration results in more rapid or accurate attainment of therapeutic Anti-Factor Xa concentrations with fewer adverse effects from over anticoagulation.

Figure. Concentration of enoxaparin at sampling intervals by syringe. The circles represent the concentration of enoxaparin. The triangles represent the high and low concentration, as per laboratory methods. X axis: The letters represent each of the 5 syringes (A, B, C, D, and E), and the number is the day of testing. Y axis: enoxaparin (mg/mL).

CONCLUSION

An 8 mg/mL dilution of enoxaparin was stable and sterile for 14 days but exhibited significant degradation at 30 days.

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 corresponding author had full access to all of the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Abbreviations ANOVA, analysis of variance; LMWH, low molecular weight heparin; NPP, normal pooled plasma

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