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ORIGINAL RESEARCH

Stability and microbiological properties of a new formulation of epoprostenol sodium when reconstituted and diluted

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Purpose: Epoprostenol, used for the treatment of pulmonary arterial hypertension (PAH), has a number of limitations related to its short half-life in aqueous solution. The aim of this study was to evaluate the stability and microbiological properties of a new formulation, namely epoprostenol sodium with arginine and mannitol excipients (epoprostenol AM; Veletri®; Actelion Pharmaceuticals Ltd, Allschwil, Switzerland).

Methods: Stability and microbiological properties of epoprostenol AM were investigated at 5°C, 25°C, and 30°C over a range of concentrations (3000–30,000 ng/mL) when reconstituted and immediately diluted with sterile water for injection (SWI) or sterile saline (sodium chloride 0.9%) for injection (SSI). Stability (change in potency over time) for up to 72 hours at 25°C and 30°C was measured immediately following dilution and after storage at 5°C. Shelf-life was assessed by determining the maintenance of potency over time relative to initial potency. For microbiological testing, diluted samples of epoprostenol AM were inoculated with a range of bacteria, yeasts, and molds for up to 14 days at 5°C or 4 days at 25°C.

Results: Epoprostenol AM reconstituted and immediately diluted to the required concentration with SWI or SSI was stable for up to 3 days at 25°C and up to 7 days at 5°C depending on the concentration. None of the diluted epoprostenol AM solutions supported microbial growth for any of the six organisms tested for up to 14 days.

Conclusions: Epoprostenol AM has improved thermal stability and does not support the growth of any microorganism tested for up to 14 days. This extended stability under ambient conditions has the potential to improve convenience for patients.

Keywords: stability, epoprostenol, formulation, microbial activity, diluents, pulmonary arterial hypertension

Introduction

Prostacyclin (prostaglandin I_2) is an important vasoactive and homeostatic mediator produced from arachidonic acid in the cell membrane of vascular endothelial cells by the action of cyclooxygenase. Prostacyclin is a potent vasodilator and possesses antithrombotic, antiproliferative and anti-inflammatory properties. These properties have led to the use of prostacyclin, in the form of the sodium salt of the synthetic exocyclic vinyl ether epoprostenol, as a treatment for pulmonary arterial hypertension (PAH), a devastating disease characterized by vasoconstriction, in situ thrombosis and remodeling of the pulmonary vessel wall.[1] However, similar to naturally occurring prostacyclin, epoprostenol hydrolyzes rapidly to 6-keto-prostaglandin F1α (6-keto-PGF1α) in a pH-dependent fashion in aqueous solutions, and the in vivo half-life of epoprostenol in humans is thought to be less than 6 minutes.[2] This hydrolytic

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lability makes the development of a robust formulation of epoprostenol for clinical use challenging.

Commercially available epoprostenol with glycine and mannitol excipients (epoprostenol GM; Flolan®; GlaxoSmithKline, Durham, NC) was approved by the US Food and Drug Administration (FDA) in 1995 but was first used to treat primary pulmonary hypertension in the early 1980s.[3] Since then a number of randomized, controlled, open-label trials of the intravenously administered drug have shown a beneficial effect on exercise capacity, hemodynamics and quality of life, as well as improvements in survival, in patients with PAH.[4–8] Based on these data, epoprostenol is recommended for the treatment of patients with moderate-to-severe PAH (World Health Organization functional groups III/IV).[9,10]

Epoprostenol GM is supplied in lyophilized vials which contain either 0.5 mg or 1.5 mg epoprostenol sodium and which must be reconstituted and diluted immediately before use using only the proprietary diluent. In solution, epoprostenol GM has limited stability at room temperature (maximum recommended: 24 hours at 2°C–8°C, or 8 to 12 hours at 25°C),[11] which necessitates refrigerated storage. Due to the short plasma half-life of epoprostenol, epoprostenol GM must be administered by continuous venous infusion via a central venous catheter for the duration of therapy. The limited stability of epoprostenol GM means that frequent changes of reservoir cartridge (≤8 hours) are required for administration at room temperature or, for administration over a 24-hour period, frozen gel packs which must be changed regularly throughout the day are necessary to ensure the solution is kept at 2°C–8°C. The need to use only the specific epoprostenol GM diluent for reconstitution, and the need for refrigeration or the use of frozen gel packs during long-term administration, leads to considerable inconvenience for the patient. In addition, abrupt interruptions of epoprostenol infusion must be avoided as, in some patients, this may lead to rebound PAH with symptomatic deterioration and may even result in death.[10] Continuity of supply is therefore critical. A formulation of epoprostenol with a better stability profile, particularly under ambient conditions, the ability to be reconstituted with commercially available intravenous diluents rather than a specific commercial diluent to improve ease of use, and the ability to be infused under ambient conditions, rather than requiring ice packs for prolonged delivery, would therefore clearly represent an improvement in convenience and, potentially, in safety.

A new product, namely epoprostenol sodium with arginine and mannitol excipients (epoprostenol AM; Veletri®; Actelion Pharmaceuticals Ltd, Allschwil, Switzerland), has been developed which is very stable and which can be reconstituted using commercially available infusion diluents. Epoprostenol AM reconstituted in the vial with 5 mL of either sterile water for injection (SWI) or sterile saline (sodium chloride 0.9%) for injection (SSI) may be refrigerated at 2°C–8°C for as long as 5 days or held at up to 25°C for up to 48 hours prior to final dilution for use.[12] The aims of the current study were to investigate the stability and microbiological properties of epoprostenol AM, immediately fully diluted following reconstitution, at 5°C and 25°C over a range of concentrations to determine the handling shelf-life when stored at the final, ready-to-use dilution. The hemolytic properties of diluted epoprostenol AM under clinical conditions were also assessed to ensure that the relatively high pH of the formulation did not have an adverse effect on blood.

Methods

Formulation

Epoprostenol AM is formulated as a sterile, lyophilized powder. In addition to epoprostenol 1.5 mg/vial as the sodium salt, the new formulation contains L-arginine US Pharmacopoeia National Formulary (USP/NF) at 50 mg/vial (buffering agent), mannitol USP/European Pharmacopoeia (EP)/Japanese Pharmacopoeia (JP) at 50 mg/vial (bulking agent), and sodium hydroxide USP/NF/EP/JP for pH adjustment.

Microbiological testing

Cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger,* and *Clostridium sporogenes* (Remel Quanti-Cult Plus; Thermo Fisher Scientific, Lenexa, KS) were prepared as per manufacturer's instructions and used within 24 hours of preparation. An initial plate count using each microbial suspension was performed to confirm appropriate level of microorganism (<10 colony-forming units [CFU]/mL) as follows: each microbial suspension was plated into sterile Petri dishes in duplicate. Using the pour plate method, bacteria were plated with tempered microbial content test agar (MCTA) and yeast and molds with tempered Sabouraud Dextrose Agar (SDA+). Plates were incubated for 3–5 days at 30°C–35°C for bacteria and 20°C–25°C for yeast and molds (*C. sporogenes* was incubated under anaerobic conditions).

Epoprostenol AM was diluted to provide 3000, 6000, 9000, 15,000, or 30,000 ng/mL samples, using either SWI or SSI, and stored in sterile glass containers. Each sterile sample of diluted epoprostenol AM was inoculated with <10 CFU/mL of each microbial suspension to simulate potential use conditions. Contents were mixed to homogenize samples. Samples

were stored for incubation for up to 96 hours at 25°C (all dilutions), or for up to 14 days at 5°C (9000 and 30,000 ng/mL) followed by 24 hours at 25°C before plating, incubation and counting. Each container was sampled at the given intervals and the number of CFU counted by using the same procedure as described above for the initial plate count. Analyses were performed in duplicate. The CFU were counted and the $\log_{10}$ reduction calculated. To allow for assay variability, "no increase" was defined as no increase in CFU > 0.5 $\log_{10}$ relative to the initial inoculum.

The test period used was chosen to reflect the stability of the product and likely clinical usage. Testing was performed at twice the expected period of stability. The period of incubation at 25°C was longer for higher concentration solutions (≥15,000) than for lower concentration solutions (≤6000 ng/mL) reflecting the lower chemical stability (and therefore shorter storage times) of low concentration solutions.

Chemical stability studies

Epoprostenol AM was reconstituted with 5 mL of either SWI or SSI. Reconstituted solutions were then immediately diluted to the final concentration (3000, 6000, 9000, 12,000 or 30,000 ng/mL) with the same diluents and stored in 50 mL CADD™ medication cassette reservoirs (polyvinyl chloride reservoir) (Smiths Medical MD Inc, St Paul, MN). Stability was measured in the medication cassette at 25°C under the following test conditions: (A) immediately following dilution at time points up to 72 hours at 25°C and 30°C, depending on concentration; (B) following 1 day storage at 5°C, and then up to 24 hours at 25°C, for 6000 ng/mL only; (C) following 7 days' storage at 5°C, and then up to 24 hours at 25°C, depending on concentration (Table 1). Testing varied for different dilutions in that some tests were not performed for particular dilutions where that dilution was known to be unstable under the conditions indicated.

Incubations were performed in dark temperature and humidity-controlled test chambers. Samples were also protected from outside humidity and light by virtue of the medication cassette. The potency over time was determined relative to initial potency for all conditions of storage, and used to determine shelf-life of diluted epoprostenol AM.

For epoprostenol AM 6000 ng/mL and 12,000 ng/mL, shelf-life after storage at 5°C was calculated by combining data collected after storage at 5°C and data collected after storage at 25°C as follows:

$$\text{Assay}_{(x \text{ days @ } 5°C + y \text{ days @ } 25°C)} = \frac{\text{Assay}_{x \text{ days @ } 5°C} \times \text{Assay}_{y \text{ days @ } 25°C}}{100}$$

Potency was determined using a high-performance liquid chromatography (HPLC) method. Epoprostenol is a challenging analyte due to the rapid hydrolysis in aqueous solutions and also due to the weak chromophor which necessitates a very low detection wavelength. HPLC was performed using a Phenomenex Gemini C18, 5 μm, 110 Å, 250 × 4.6 mm column (Phenomenex, Torrance, CA) where the conditions were as follows: autosampler temperature 5°C ± 3°C; column temperature 25°C ± 3°C; flow rate 1.0 mL/min; mobile phase A 80% 25 mM pH 9.0 borate buffer/20% acetonitrile (v/v) and B 50% water/50% acetonitrile (v/v), gradient–time 0 100% A/0% B, time 15.01 minutes and 25.01 minutes 0% A/100% B, time 30 minutes 100% A/0% B; injection volume 20 μL; retention time of epoprostenol peak was approximately 10 minutes; chromatographic run time 30 min; detection wavelength: 205 nm. Reference standard was epoprostenol

Table 1 Stability protocol[a]

Concentration	Time interval/storage conditions		
	Condition A	**Condition B**	**Condition C**
3000 ng/mL	0, 8, 12, 24 hours at 25°C 0, 8, 12, 24 hours at 30°C	nd	nd
6000 ng/mL	0, 8, 12, 24 hours at 25°C 0, 8, 12, 24 hours at 30°C	0, 24 hours at 5°C plus 0, 12 hours at 25°C	nd
9000 ng/mL	0, 24 hours at 25°C 0, 24 hours at 30°C	nd	0, 7 days at 5°C plus 12 hours at 25°C
12,000 ng/mL	0, 24, 48, 72 hours at 25°C 0, 24, 48, 72 hours at 30°C	nd	nd
30,000 ng/mL	0, 24, 48, 72 hours at 25°C 0, 24, 48, 72 hours at 30°C	nd	0, 7 days at 5°C plus 24 hours at 25°C

Notes: [a]Conditions tested varied by dilution based on the known stability of diluted epoprostenol AM; condition A: tested immediately following dilution at time points up to 72 hours at 25°C and 30°C, depending on concentration; condition B: tested following 1 day storage at 5°C and then up to 24 hours at 25°C, for 6000 ng/mL; condition C: tested following 7 days' storage at 5°C and then up to 24 hours at 25°C, depending on concentration.
Abbreviations: AM, arginine and mannitol; nd, not done.

as the sodium salt (SAI Advantium Pharma Ltd, Hyderabad, India). The method has been validated with respect to specificity, range (accuracy, linearity, precision) for both epoprostenol and related substances, limit of detection, limit of quantitation, relative response factor for 6-keto-PGF1α, robustness and solution stability.

Levels of related substances were also determined. Unidentified peaks were only designated as "true" degradation products if they occurred at consecutive time points or at the last time point of the respective study. Due to the low detection wavelength, minute impurities originating, for instance, from dissolved air or the eluent giving rise to such spurious peaks are easily detected. Shelf-life was assessed by determining the period of time over which a potency greater than or equal to 90% of the initial value measured following dilution and prior to storage (time 0) was maintained. However, slightly lower potency was also considered acceptable, particularly for solutions with low epoprostenol AM concentrations for two reasons: (1) upon validation standard deviation of the method was found to be higher relative to the higher concentrations, probably due to the greater number of dilution steps and (2) in clinical practice low concentration solutions are used during the treatment initiation phase, where a patient's response to the drug is closely monitored in a hospital setting and the dose can be adjusted if needed. For these reasons, a remaining potency of ≥86% compared with initial potency was considered to be an acceptable level without compromising patient safety.

The pH of diluted solutions, taken at each study point was conducted using a PerpHect Ross Sure-Flow Combination pH electrode (Thermo Scientific, Barrington, IL). Changes in physical appearance or particulate matter were examined by observing the samples in clear glass containers against a black background by eye.

Assessment of hemolytic properties

Whole human blood from an adult volunteer was collected into standard vials containing sodium heparin anticoagulant and used for testing on the same day. A 1.5 mg vial of epoprostenol AM was reconstituted and diluted to give a final concentration of 15,000 ng/mL using SSI and SWI. A 70 µL aliquot of each solution was added to 5 mL of blood. This ratio was selected based on estimates of the infusion rate of the diluted product and peripheral blood flow in the forearm. The infusion rate of the diluted product was estimated to be ~0.05 mL (50 µL) per pulse of the pump; therefore, a slightly higher value (70 µL) was selected as the worst case scenario. The blood flow rate in the forearm has been calculated to be 3–30 mL/minute.[13] At the highest dosing rate, one pulse is delivered every 30 seconds; therefore, at the slowest blood flow, 50 µL of diluted product would contact ~1.5 mL of blood. However, as epoprostenol AM is generally administered via a central venous catheter, the volumetric blood flow rate would be much higher, and so dilution of the product will be greater. Nevertheless, the final dilution tested was estimated as a worse case volume to simulate delivery into a peripheral vein, as temporary peripheral infusion may be used until central access is established. Samples were incubated at 37°C in a water bath, and then centrifuged for 10 minutes at 3000 rpm (2095 × g) at ambient temperature. All preparations were made in triplicate. For comparison, samples containing glycine buffer diluents (Flolan® diluent; GlaxoSmithKline), 60 µg/mL sodium hydroxide in normal saline, and pH 13-adjusted normal saline were prepared in the same way. Samples of whole blood only were used to monitor the hemolytic impact of the procedure.

Two methods were used to estimate the hemolysis. In the first, the values were compared to a hemoglobin standard. A standard curve for hemoglobin concentration was created as per the ASTM Protocol F756.[14] Samples were treated with cyanmethemoglobin (CMH) reagent for spectroscopic measurement (absorbance 540 nm; Hewlett Packard 8435 UV-Visible spectrophotometer with 1 cm quartz cuvette) to reduce all the forms of hemoglobin. Percent hemolysis was calculated by conversion of spectrophotometric absorbance to hemoglobin values using the standard curve and dividing by the total blood hemoglobin level for the blood sample used. In the second method, the hemolysis in the samples was compared to controls of 1:1 blood:normal saline (0% hemolysis) and 1:1 blood:deionized water (100% hemolysis) using a procedure adapted from the literature.[13–15] Absorbance was measured at 540 nm and % hemolysis calculated by dividing the sample absorbance value by the absorbance value representing 100% hemolysis.

Results

Microbiological findings

No increase, but a real reduction, with respect to initial inoculum of any tested organism was seen in any diluted sample (ranging from 3000 to 30,000 ng/mL) of epoprostenol AM in either diluents over the full course of the study (48–96 hours depending on dilution at 25°C; 14 days at 5°C) (Tables 2 and 3). A few results for *A. niger* and *C. albicans* appeared to be static with all below the level defined as growth (0.5 $\log_{10}$).

Table 2 Preservative effectiveness testing for epoprostenol AM stored at 25°C (reduction in count[a])

Time (hours)	Sterile water for injection						Sterile saline (sodium chloride 0.9%) for injection					
	S. aureus	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*	*C. sporogenes*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*	*C. sporogenes*
	3000 ng/mL											
12	0.48 log	>0.60 log	0.55 log	–0.25 log	–0.18 log	>0.30 log	>0.60 log	>0.48 log	>0.90 log	0.18 log	–0.40 log	0.30 log
24	>0.48 log	>0.60 log	0.85 log	–0.10 log	–0.40 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	0.18 log	–0.40 log	>0.30 log
36	>0.48 log	>0.60 log	0.85 log	0.60 log	0.00 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	0.48 log	0.30 log	–0.18 log
48	>0.48 log	>0.60 log	0.85 log	0.00 log	–0.30 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	0.48 log	0.30 log	0.30 log
	6000 ng/mL											
12	>0.48 log	>0.60 log	0.85 log	0.12 log	–0.18 log	>0.30 log	>0.60 log	>0.48 log	>0.90 log	0.18 log	0.30 log	–0.18 log
24	>0.48 log	>0.60 log	>0.85 log	0.12 log	0.30 log	0.00 log	>0.60 log	>0.48 log	>0.90 log	0.18 log	0.30 log	0.30 log
36	>0.48 log	>0.60 log	>0.85 log	>0.60 log	0.30 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	0.18 log	>0.30 log	0.30 log
48	>0.48 log	>0.60 log	>0.85 log	0.12 log	0.00 log	>0.30 log	>0.60 log	>0.48 log	>0.90 log	0.48 log	>0.30 log	0.30 log
	15,000 ng/mL											
24	0.3 log	1.3 log	0.6 log	0.1 log	0.00 log	nd	>0.9 log	>1 log	>1 log	0.2 log	0.00 log	nd
48	1.0 log	>1 log	1.0 log	0.7 log	0.00 log	nd	>1 log	>1 log	>1 log	0.2 log	0.00 log	nd
72	>1 log	>1 log	>1 log	>1 log	0.00 log	nd	>1 log	>1 log	>1 log	0.5 log	0.00 log	nd
96	>1 log	>1 log	>1 log	>1 log	0.00 log	nd	>1 log	>1 log	>1 log	0.8 log	0.00 log	nd
	30,000 ng/mL											
24	>0.48 log	>0.60 log	>0.85 log	>0.60 log	0.00 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	0.48 log	0.00 log	0.30 log
48	>0.48 log	>0.60 log	>0.85 log	>0.60 log	0.00 log	0.30 log	>0.60 log	>0.48 log	>0.90 log	>0.48 log	0.30 log	>0.30 log
72	>0.48 log	>0.60 log	>0.85 log	>0.60 log	–0.30 log	>0.30 log	>0.60 log	>0.48 log	>0.90 log	>0.48 log	0.00 log	>0.30 log
96	>0.48 log	>0.60 log	>0.85 log	>0.60 log	–0.18 log	>0.30 log	>0.60 log	>0.48 log	>0.90 log	>0.48 log	–0.18 log	>0.30 log

Notes: [a]Reduction in the number of CFU relative to the initial inoculation. Negative numbers indicate an increase in CFU over the initial inoculum. 'No increase' was defined as no increase in CFU > 0.5 $\log_{10}$.
Abbreviations: AM, arginine and mannitol; CFU, colony-forming units; nd, not done.

Table 3 Preservative effectiveness testing for epoprostenol AM stored at 5°C (reduction in count[a])

Time (days)	Sterile water for injection					Sterile saline (sodium chloride 0.9%) for injection				
	S. aureus	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*	*C. sporogenes*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*	*C. sporogenes*
9000 ng/mL												
4	>0.90 log	>0.70 log	>0.70 log	–0.12 log	0.48 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	0.00 log	0.00 log
7	>0.90 log	>0.70 log	>0.70 log	0.18 log	0.08 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	0.10 log	0.00 log
10	>0.90 log	>0.70 log	>0.70 log	>0.48 log	0.08 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	0.70 log	0.00 log
14	>0.90 log	>0.70 log	>0.70 log	>0.48 log	0.30 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	>0.70 log	0.00 log
30,000 ng/mL												
4	>0.90 log	>0.70 log	>0.70 log	0.18 log	0.78 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	0.40 log	0.00 log
7	>0.90 log	>0.70 log	>0.70 log	>0.48 log	0.18 log	–0.30 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	0.10 log	0.00 log
10	>0.90 log	>0.70 log	>0.70 log	>0.48 log	0.00 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	>0.70 log	0.00 log
14	>0.90 log	>0.70 log	>0.70 log	>0.48 log	0.08 log	0.00 log	>0.85 log	>0.30 log	>0.85 log	>0.70 log	>0.70 log	0.00 log

Notes: [a]Reduction in the number of CFU relative to the initial inoculation. Negative numbers therefore indicate an increase in CFU over the initial inoculum. "No increase" was defined as no increase in CFU > 0.5 $\log_{10}$.
Abbreviations: AM, arginine and mannitol; CFU, colony-forming units.

Potency of diluted epoprostenol AM over time

The potency of epoprostenol AM 3000 ng/mL–30,000 ng/mL during storage at 25°C and 30°C following immediate dilution (test condition A) is shown in Table 4 and Table 5, respectively.

At 25°C, potency of epoprostenol AM was >90% up to 24 hours at concentrations between 9000 ng/mL and 12,000 ng/mL, and 72 hours at 30,000 ng/mL. Acceptable levels of potency (≥86%) were seen up to 12 hours at 25°C for epoprostenol AM 3000 ng/mL and 24 hours for epoprostenol AM 6000 ng/mL. Loss of potency was higher at 30°C compared with 25°C for all dilutions tested. Additional loss in potency did not exceed 0.2% per hour at 30,000 ng/mL, 0.3% at 12,000 and 9000 ng/mL, 0.5% at 6000 ng/mL, and 1% at 3000 ng/mL. No relevant increase in the level of any unknown impurity was observed under any condition tested. No change in physical appearance or any visible particulate matter was observed.

Potency of diluted epoprostenol AM following storage at 5°C

Test conditions and potency of epoprostenol AM following storage at 5°C following immediate dilution is shown in Table 6. Potency data after 1 day storage at 5°C were unavailable for epoprostenol AM 12,000 ng/mL; therefore, the data obtained with epoprostenol AM 6000 ng/mL were used to calculate final potency under test condition B. Since stability was shown to be lower at lower concentrations, this therefore represents a "worst case" scenario. Based on this, potency of epoprostenol AM 12,000 ng/mL under test condition B (1 day at 5°C plus 12 hours at 25°C) was calculated to be ≥90% for both diluents. No data were available for test condition C

Table 4 Potency of epoprostenol AM 3000–30,000 ng/mL stored at 25°C following immediate dilution

Epoprostenol AM, ng/mL	Diluent	Potency over time (hours), %				
		0	8	12	24	72
3000	SWI	100	92	88	75	nd
	SSI	100	91	86	72	nd
6000	SWI	100	96	93	87	nd
	SSI	100	94	93	86	nd
9000	SWI	100	nd	nd	90	nd
	SSI	100	nd	nd	90	nd
12,000	SWI	100	nd	nd	91	nd
	SSI	100	nd	nd	93	nd
30,000	SWI	100	nd	nd	97	91
	SSI	100	nd	nd	97	90

Abbreviations: AM, arginine and mannitol; SWI, sterile water for injection; SSI, sterile saline (sodium chloride 0.9%) for injection; nd, not done.

Table 5 Potency of epoprostenol AM 3000–30,000 ng/mL stored at 30°C following immediate dilution

Epoprostenol AM, ng/mL	Diluent	Potency over time (hours), %				
		0	8	12	24	72
3000	SWI	100	86	79	58	nd
	SSI	100	84	76	55	nd
6000	SWI	100	92	88	77	nd
	SSI	100	92	88	76	nd
9000	SWI	100	nd	nd	83	nd
	SSI	100	nd	nd	82	nd
12,000	SWI	100	nd	nd	86	nd
	SSI	100	nd	nd	87	nd
30,000	SWI	100	nd	nd	93	81
	SSI	100	nd	nd	92	79

Abbreviations: AM, arginine and mannitol; SWI, sterile water for injection; SSI, saline (sodium chloride 0.9%) for injection; nd, not done.

(7 days at 5°C plus 12 hours at 25°C) for epoprostenol AM 12,000 ng/mL; therefore, the same recommendation made for epoprostenol AM 9000 ng/mL was applied to calculate shelf-life (worst case scenario). Potency of epoprostenol AM 30,000 ng/mL under test condition C (7 days at 5°C plus 24 hours at 25°C) was ≥90% for both diluents. Furthermore, data show that epoprostenol AM 30,000 ng/mL when held at 25°C demonstrates stability for up to 72 hours; therefore, since storage at 25°C is the worst case scenario compared with storage at 5°C, the potency should still be acceptable at this dilution for up to 48 hours at 25°C after storage for 1 day (24 hours) at 5°C. No relevant increase in the level of any unknown impurity was observed under any condition tested.

Table 6 Potency of epoprostenol AM 6000–30,000 ng/mL after storage at 5°C following immediate dilution

Concentration of diluted solution	Diluent	Storage conditions	Potency, %
6000 ng/mL	SWI	24 hours at 5°C 12 hours at 25°C	92
	SSI	24 hours at 5°C 12 hours at 25°C	92
9000 ng/mL	SWI	7 days at 5°C 12 hours at 25°C	91
	SSI	7 days at 5°C 12 hours at 25°C	87
12,000 ng/mL	SWI	24 hours at 5°C 24 hours at 25°C	91[a]
	SSI	24 hours at 5°C 24 hours at 25°C	92[a]
30,000 ng/mL	SWI	7 days at 5°C 24 hours at 25°C	93
	SSI	7 days at 5°C 24 hours at 25°C	90

Notes: [a]Extrapolated data. Potency was extrapolated using data from 6,000 ng/mL.
Abbreviations: AM, arginine and mannitol; SWI, sterile water for injection; SSI, sterile saline (sodium chloride 0.9%) for injection.

No change in physical appearance or any visible particulate matter was observed.

pH of diluted solutions

Overall (at 5°C or 25°C), pH of the diluted solutions ranged from 9.9–11.3, with the lowest pH in the 3000 ng/mL solution and the highest in the 30,000 ng/mL solution. Variation in pH of the diluted solutions was minimal during storage and did not show any temporal pattern. pH ranges were as follows: 3000 ng/mL 9.9–10.4 (SWI) and 9.9–10.2 (SSI); 6000 ng/mL 10.2–10.4 (both diluents); 30,000 ng/mL 11.0–11.3 (SWI) and 10.7–11.2 (SSI) .

Assessment of hemolytic properties

Using the method 1 (the CMH procedure), minimal hemolysis was noted for epoprostenol AM reconstituted in SWI (0.2%) and no measurable hemolysis was observed for epoprostenol AM reconstituted in SSI. Similarly, using the second (normal saline procedure), epoprostenol AM hemolysis of epoprostenol AM reconstituted in SWI was 0.1%, and no measurable hemolysis was observed for epoprostenol AM reconstituted in SSI. Hemolysis was ≤0.3% for comparator samples. The blood only (no added test materials) produced very similar results (Table 7).

Discussion

Due to its limited stability, epoprostenol GM must be reconstituted and diluted immediately prior to clinical use, with the need for frequent changes of reservoir cartridge if administered at room temperature, or the use of frozen gel packs which must be changed regularly throughout

Table 7 Relative hemolysis of epoprostenol formulations

Diluent	Hemolysis (%) CMH procedure	Hemolysis (%) Normal saline procedure
Epoprostenol AM reconstituted in SWI	0.2	0.1
Epoprostenol AM reconstituted in SSI	0.0	0.0
Flolan diluent (placebo)	0.1	0.0
Normal saline adjusted with 60 µg NaOH/100 mL saline	0.0	0.0
Normal saline adjusted to pH 13	0.3	0.1
Plain blood (control)	0.0	0.0

Abbreviations: AM, arginine and mannitol; SWI, sterile water for injection; SSI, sterile saline (sodium chloride 0.9%) for injection; NaOH, sodium hydroxide; CMH, cyanmethemoglobin.

the day to ensure the solution is kept at 2°C–8°C.[11] In addition, epoprostenol GM must be reconstituted using a specific commercial diluent only, adding to the inconvenience of treatment.[11] In the current study, epoprostenol AM fully diluted in either SWI or SSI (3000 to 30,000 ng/mL) demonstrated stability of potency for between 12 and 72 hours at 25°C, depending on concentration, and up to 24 or 48 hours, depending on concentration, after storage at 5°C for 1 or 7 days, respectively. Furthermore, epoprostenol AM fully diluted in SWI or SSI was self-preserving, with no increase in inoculated microorganisms when held at 25°C for up to 48 hours, or at 5°C for up to 14 days, well beyond the recommended shelf-lives for the diluted product. This self-preservation is likely to result from the high pH of the amino acid buffer used in epoprostenol AM (10.0–12.5 depending on dilution). Testing included a range of microorganisms representing a broad spectrum of potential manufacturing, nosocomial and household contaminants, including Gram-negative and Gram-positive bacteria, common fungi, and molds. Micrococcus was not included in the present study; however, given that it is an important pathogen associated with bloodstream infections in patients receiving epoprostenol,[16] it will be included in future testing.

In the current study, epoprostenol AM showed a high degree of stability at 25°C, which varied depending on concentration and conditions of use, but was not markedly affected by diluents used. Stability, and so shelf-life, is generally assessed by determining the period of time over which a potency greater than or equal to 90% is maintained; however, slightly lower potency was considered acceptable, specifically for solutions with low epoprostenol AM concentrations where the relative standard deviation of the method is highest. In addition, low concentrations of epoprostenol are used during the treatment initiation phase, where patients' response to the drug is closely monitored in a hospital setting and the dose is adjusted if needed. For these reasons, a remaining potency of ≥86% was considered to be an acceptable level without compromising patient safety. Overall, higher concentrations of epoprostenol AM showed greater stability under all test conditions, given that these higher concentrations present the higher pH.

Based on the findings of this study, fully diluted epoprostenol AM would be acceptable for use at 25°C for up to 24 hours at concentrations between 6000 ng/mL and less than 30 000 ng/mL, and for up to 72 hours at concentrations of 30,000 ng/mL and above this. Following previous storage for 1 day at 5°C, 6000 ng/mL epoprostenol AM maintained acceptable activity for up to 12 hours at 25°C. Following previous storage for 7 days at 5°C, epoprostenol AM maintained acceptable activity for up to 24 hours at 25°C depending on dilution. Although potency decreased at a greater rate in solutions stored at 30°C, it is considered that short excursions at this temperature should not have a significant impact on the overall loss of potency, particularly at concentrations above 6000 ng/mL, where the additional loss of potency at 30°C relative to 25°C did not exceed 0.5% per hour. In all solutions tested and under all conditions, no relevant increases in related substances or impurities were seen. The major hydrolytic metabolite of epoprostenol, 6-keto-PGF1α, was present but this is not considered relevant as it is also a metabolite of endogenous prostacyclin and substantially less active than the parent molecule.

The high pH of the reconstituted solution is likely to be a major contributor to this improved stability. The vinyl ether moiety of epoprostenol is best stabilized in solution formulations by buffering under basic conditions (>pH 8.8), and high pH solutions (>pH 10) have been used to stabilize prostacyclin, for example, to enable extraction from whole blood.[17] The increasing stability of prostacyclin with increasing pH may be related to the reduced levels of hydronium ions in more alkaline solutions, as the hydrolysis of epoprostenol is hydronium ion-catalyzed.[18] The pH of reconstituted epoprostenol AM is around 12.5, and ranged from around 9.9–11.3 when diluted depending on concentration, with a lower pH at lower concentrations. This compares with a range of 10.2–10.8 for epoprostenol GM. The narrower range of pH seen with epoprostenol GM is due to the use of the proprietary buffered diluent compared with the use of nonpropriety SWI/SSI for epoprostenol AM, which will result in wider variation in pH range. The higher pH of reconstituted epoprostenol AM reflects the chemical structure of the two amino acids used as buffers, arginine in epoprostenol AM and glycine in epoprostenol GM. The guanidine group of arginine has a particularly high pK_a (13.2), whereas the highest pK_a value for the α-amino group of glycine is 9.8. Therefore arginine is surprisingly more suitable for providing a high alkaline environment and a better pH control upon dilution in IV diluents. The rapid hydrolysis of epoprostenol is catalyzed by specific hydronium ions. The observed rate constant can be represented by the equation:

$$k_{obs} = k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-]$$

where k_{obs} is overall pseudo-first order rate constant, and $k_{H_3O^+}$ and k_{OH^-} are rate constants for specific hydronium ion and hydroxide ion, respectively. As pH increases, the contribution

Table 8 Recommended shelf-life at 25°C of diluted epoprostenol AM

Concentration range	Immediate use after dilution[a]	After storage for 1 day at 5°C	After storage for 7 days at 5°C
≥3000–<6000 ng/mL	12 hours	Do not use	Do not use
≥6000–<9000 ng/mL	24 hours	12 hours	Do not use
≥9000–<12,000 ng/mL	24 hours	12 hours	12 hours
≥12,000–<30,000 ng/mL	24 hours	24 hours	12 hours
≥30,000 ng/mL	72 hours	48 hours	24 hours

Note: [a]In either sterile water for injection or sterile saline (sodium chloride 0.9%) for injection.
Abbreviation: AM, arginine and mannitol.

of the specific hydronium ion catalysis diminishes and hence stability is improved.

Several agents with high pH formulations are approved for intravenous use, although such formulations may potentially have biocompatibility issues. However, epoprostenol AM does not exhibit these issues because it is administered slowly via a central catheter and in extremely small quantities. Thus, there is considerable dilution in the blood, and so a reduced potential from problems such as hemolysis. Moreover, the concentration of the basic amino acid is at a level to maintain the pH, but is also low enough to ensure the buffer capacity of the blood is sufficient to overcome the pH of the slowly delivered medication. In fact, this study showed no difference in hemolysis between epoprostenol AM diluted with either SWI or SSI at concentrations and dilutions chosen to reflect clinical dose and normal blood flow. In addition, the low degree of hemolysis seen in this study (≤0.2%) is nearly 100-fold less than the values recommended by recent guidelines, which consider formulations with hemolysis values of <10% to be nonhemolytic.[13]

Although a higher pH would be expected to improve the stability of epoprostenol, it is likely that the extended stability of epoprostenol AM relative to epoprostenol GM is not solely due to this effect, but also due to the other features of arginine (high buffer capacity) as compared with glycine. Arginine is widely used to prevent protein degradation in aqueous solution because of its ability to suppress protein aggregation, although the exact mechanism by which it achieves this is unclear.[19] It is theoretically possible that the improved stability of epoprostenol AM may be linked to the presence of multiple ionic binding sites on the arginine molecule (guanidine amine and secondary amine) that could potentially form an ionic cluster with multiple molecules of epoprostenol and therefore create a sterically protective environment and so decrease the rate of hydrolysis.

Recommendations for the shelf-life of fully diluted epoprostenol AM stored in the medication cassette based on the microbiological and stability data obtained in this study are shown in Table 8.

Conclusion

Epoprostenol AM has improved thermal stability, allowing it to be fully diluted immediately after reconstitution and stored ready for use for up to 72 hours at 25ºC, depending on concentration, using readily available intravenous diluents. This extended stability under ambient conditions has the potential to improve convenience for patients, with long-term administration requiring change of medication cassettes every 24 hours for concentrations above 3,000 ng/mL, and without the need for ice packs. Fully diluted epoprostenol AM is self-preserving, and did not support the growth of any microorganism tested for up to 48 hours at room temperature or 14 days when refrigerated. This product has been successfully and safely administered to human patients in clinical studies[20] and has been available commercially for more than 2 years.

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Disclosure

OL and DB are employees of Actelion Pharmaceuticals Ltd, the manufactures of Veletri®. LW-L is a former employee of GeneraMedix Inc, the company who developed the drug formulation. RI is an employee of TPM Laboratories, Inc, the testing laboratory contracted to conduct the analytical chemistry testing. NP is the principal inventor of this formulation developed at SciDose LLC. GeneraMedix Inc, licensed this formulation from SciDose LLC and all further development studies were conducted in collaboration with GeneraMedix Inc. Actelion

Pharmaceuticals Ltd, then purchased the rights to the product from GeneraMedix Inc.

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