Production Standard and Stability of Compounded Del Nido Cardioplegia Solution

Hospital Pharmacy
2017, Vol. 52(11) 766–773
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DOI: 10.1177/0018578717738510
journals.sagepub.com/home/hpx

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Abstract

Background: The del Nido cardioplegia solution (dNCS) was originally developed for pediatric cardiac surgery, being now also used for adult patients. Hospital pharmacies frequently resort to internal dNCS production which has led to an increase in the need for validated parameters for compounding and storage. **Objective:** This report defines in-house production standards, as well as the stability of dNCS under optimal storage conditions. **Methods:** All ingredients were sterile and United States Pharmacopeia (USP)/National Formulary (NF) certified. All final bags were quarantined at 4°C for quality control, when 3 of 33 weekly bags were randomly assayed for potassium content. Each lot was only released if all 3 samples were within $\pm 5\%$ of target. Stability testing was performed per USP 797 guidance. Over a 6-month period, 4 different lots and 4 bags from each lot of dNCS were assayed. Each bag was assessed for physical and chemical stability while refrigerated at 4°C, at 35°C in an incubator, and at 70°C under 80% relative humidity. A light exposure arm was also set up at 25°C under 150 lumens. Calibrators of lidocaine, mannitol, and gluconate were freshly prepared and assayed with the samples by Liquid chromatography/Mass spectrometry (LC/MS). **Results:** Lidocaine concentrations averaged 0.117 mg/mL (95.8% of theoretical) at 4°C for 30 days. At 35°C, they decayed by 67% in 30 days, while at 70°C nearly 50% was lost after the first day. A first-order kinetics was observed with an Arrhenius activation energy of 25 kcal/mol. Degradation products identified under stress conditions were absent in the stable product. **Conclusions:** The dNCS is stable for at least 30 days under 4°C refrigeration in ethylene vinyl acetate (EVA) bags.

Keywords

del Nido cardioplegia, cardiac surgery, sterile products compounding, hospital pharmacy, cardioplegia additive stability

Introduction

An ideal cardioplegia solution affords safe and sustained electromechanical cardiac quiescence during cardiac surgery when the heart must be stopped. It must also provide a stable arrest while minimizing electrolyte shifts across the myocardial cell membrane, particularly for calcium, avoiding adverse effects once the heart is reperfused. Due to stability issues and individual physician preferences, hospital pharmacies often compound complex sterile solutions such as del Nido cardioplegia solution (dNCS). This practice is not without risk¹ and strict state and federal compounding guidelines have been generated over the years.

In the 1990s, the del Nido cardioplegia formula was developed for use in infants and children undergoing cardiac surgery.² By using hypothermia and specific additives in the solution, cardioplegic arrest is able to induce a reduction in myocardial energy consumption.^{3,4} The usage of the dNCS has been common practice in pediatric cardiac surgery for decades now,² with literature reports comparing it against institutional standard multidose cardioplegia protocols.^{5,6} The use of dNCS in adult cardiac surgery has increased too

over the last several years, driven by its safety and efficacy^{3,7} despite limited animal experimentation and controlled trials in adult cardiac surgery patients. Early reports of del Nido cardioplegia in adult cardiac surgery are encouraging with less interventions required by the surgeon owing to longer intervals between cardioplegia redosing and improved glucose management with decreased postoperative insulin requirements.⁸

The widespread and increasing use of dNCS necessitates formal and validated compounding methods. Stability

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Table 1. Supplies for Del Nido Cardioplegia Solution Preparation (Weight/Volume [w/v]).

Supplies	Initial concentration	Volume (mL)
Plasma-Lyte A 7.4 ^a	—	1000 (1 bag)
Sodium bicarbonate USP	8.4% w/v (1 mEq/mL)	13
Mannitol USP	20% w/v (0.2 g/mL)	16.3
Magnesium sulfate USP	50% w/v (0.5 g/mL, 4 mEq/mL)	4
Lidocaine USP	1% w/v (0.01 g/mL)	13
Potassium chloride USP	2 mEq/mL	13
Final compounded volume		1059

^aBaxter Healthcare Corporation, Deerfield, Illinois.

testing under ideal storage conditions, as outlined in USP 797⁹ also needs to be investigated. This report aims to satisfy both needs.

The del Nido modified depolarizing cardioplegia solution is a crystalloid-based preparation containing sodium bicarbonate, mannitol, lidocaine, magnesium sulfate, and potassium as additives in a balanced electrolyte solution. The sodium bicarbonate acts as a pH buffer to assist with excess hydrogen ions in the arrested heart; the mannitol is a free radical scavenger which also maintains osmotic pressure; magnesium sulfate prevents contractile activation by acting as a competitive calcium channel blocker; lidocaine in local high dose, acts as a sodium channel blocker, preventing electrical activity and promoting spontaneous defibrillation; and potassium chloride depolarizes the myocardial cell membrane.² The final product intended for coronary perfusion is used in a ratio of 4 parts crystalloid solution to 1 part patient whole blood, and is most often administered antegrade in a cold blood and single-dose fashion.²

Hospital Pharmacy Compounding Process

All compounding takes place in an ISO 5 (ie, laminar airflow hood) located within an ISO 7 buffer area. All ingredients are USP/NF-certified commercially available products, and no nonsterile ingredients are used during the compounding process (Table 1).

All compounding manipulations were performed by certified pharmacy technicians using appropriate aseptic technique under the continuous direct supervision of a registered pharmacist. Each ingredient was sequentially transferred into sterile empty 1-liter *ethylene vinyl acetate* (EVA) containers using the EXACTAMIX automated compounding system (Baxter Healthcare Corporation, Englewood, Colorado) per the manufacturer's instructions. As this compounder is also used for the preparation of parenteral nutrition solutions, prior to beginning the compounding process, a special configuration for ingredient placement on the device specific to the cardioplegia preparation was activated on the system. As an additional safety measure, each ingredient was bar coded and scanned to match with its position on

the compounder. In the case of lidocaine 1%, the solution was repackaged from its original source container into a sterile, 60-mL syringe and labeled with appropriate bar coding and checked by a registered pharmacist prior to placement on the compounder. Once the device was set up, the compounder was calibrated and checked by a registered pharmacist. Each sterile empty EVA bag was labeled prior to placement on the compounder using labels generated by the Abacus software (Baxter Healthcare Corporation, Englewood, Colorado) program that interfaces with the EXACTAMIX compounder. Each bag was individually compounded and weighed at the completion of the compounding cycle, and compared with the calculated final expected weight. Only bags whose final weights were within a $\pm 2\%$ margin were considered acceptable and all others would be discarded. In addition to observing the entire compounding process as it occurred, pharmacists also visually inspected the final product for the correct volume and any evidence of visible particulate matter. Furthermore, the pharmacist reviewed the automated report generated by the compounder for any additive delivery malfunction (ie, formation of bubbles), source container changes, and confirmation that the weight of each bag was within the desired $\pm 2\%$ range. Both pharmacist and technician signed the manufacturing log and product label associated with the final compounded product. All bags were stored under refrigeration at 4°C in a dedicated quarantine refrigerator until the quality control (QC) results, described below, were known. Given our high cardiac surgery case volume, a batch of 33 bags is usually compounded weekly during a single compounding period.

As part of the in-process QC, 3 of the 33 weekly bags are randomly selected postproduction and assayed for potassium content as an indicator. A sample of 5 mL of dNCS was aseptically withdrawn using a 10-mL syringe, which was capped and labeled with the bag serial number and preparation date. It was then sent to the institution's clinical chemistry laboratory for analysis. As per the agreement with the laboratory, all samples were batch processed and the results were reported to the pharmacy within 90 minutes of reception. A different pharmacist not involved in the compounding process reviewed the laboratory results prior to the release of each batch of dNCS to the surgical team. The laboratory

Cardioplegia Solution
Plasma-Lyte A - 1000 mL
Sodium Bicarbonate 1 mEq/mL - 13 mL
Mannitol (20%)- 16.3 mL
Magnesium Sulfate (50%)- 4 mL
Lidocaine (1%)- 13 mL
Potassium Chloride 2 mEq/mL - 13 mL

Final Volume = 1059.3 mL
EXPIRATION DATE: (9 days from current date)
Keep refrigerated - DO NOT FREEZE
Dr. del Nido Formula
CARDIOPLEGIA ADMIXTURE SOLUTION
NOT FOR I.V. ADMINISTRATION
CARDIAC PERFUSION ONLY

Figure 1. Cardioplegia solution label.

results were ultimately filed with the respective compounding records and retained for 2 years per departmental policy. The 30 bags of cardioplegia were only released if all 3 lab samples reported a potassium concentration within $\pm 5\%$ of the expected value (ie, 26 mEq/L). If any of the laboratory results were outside this margin of tolerance, the batch would not be released and the suspect bags would be resampled and retested to rule out any laboratory error. If the results were still out of range, a new batch of cardioplegia solutions would be compounded and tested according to the exact same procedure. To date, all batches of cardioplegia using this process have been within the desired range for weight, volume, and potassium content, and it has not been necessary to reject a production batch.

Once all protocolled QC tests have been completed, a final product label (Figure 1) was placed on each bag of cardioplegia, making sure that neither the incremental volume marking on the bag nor the Abacus bag label was obscured. Only the original expiration date/time generated by the Abacus bag label was covered by the new label. In addition, "refrigerate" and "cardioplegia solution" auxiliary labels were also affixed to each bag. A beyond use date of 9 days was assigned to each batch of dNCS, with a labeled expiration date of 7 days to account for the usual quarantine time prior to release per our production standard.

Stability Testing

Stability testing was performed per USP 797 guidance documents regarding stability testing.^{10,11} Stock dNCS bags were obtained from the Pharmacy Department at Boston Children's Hospital, as they were regularly compounded for clinical use. Over a 6 month period, 4 different lots of dNCS, and 4 bags from each lot, were assayed. Given that stability data

are lacking for several of the additives in dNCS, this study was conducted at different environmental conditions to establish an evidence-based shelf-life. Each dNCS bag was assessed for physical and chemical stability under 3 different environments, ie, refrigerated at 4°C, at 35°C in an incubator, and at 70°C under 80% relative humidity in an environmental chamber. A light exposure arm was also set up at 25°C under 150 lumens, paired with a similar sample in the dark at the same temperature.

The Plasma-Lyte A 7.4 (Baxter Healthcare Corporation, Deerfield, Illinois) solution used in dNCS contains no antimicrobials and is adjusted with sodium hydroxide by the manufacturer to a pH of 7.4 (reported range, 6.5-8.0). The electrolyte content of the salt components per 100 mL is as follows: 526 mg sodium chloride, 502 mg sodium gluconate, 368 mg sodium acetate trihydrate, 37 mg potassium chloride, and 30 mg magnesium chloride. Total ionic concentrations are 140 mEq/L sodium, 5 mEq/L potassium, 3 mEq/L magnesium, and 98 mEq/L chloride. For the purpose of this chemical stability testing, lidocaine, mannitol, and gluconate concentrations were monitored over time.

Using a 1-mL syringe, 1 mL of dNCS sample was drawn at each sampling time from the original bag and transferred to a 1.5-mL snap cap polypropylene Eppendorf centrifuge tube. Immediately after, 100 μ L of that dNCS sample were taken and diluted with 900 μ L of pure milliQ water; 10 μ L of the diluted solution were mixed with 10 μ L of internal standard (IS) at 1 mg/mL and diluted with 980 μ L of pure milliQ water in a 1.5-mL screw cap amber glass injection vial to be loaded onto the liquid chromatography (LC) sample tray for injection. Freshly made calibrators and QC samples were run in the same sequence with the unknown samples to construct a calibration curve and establish the accuracy of the results.

Liquid Chromatography

An Agilent 1200 series high-performance liquid chromatography system (Agilent Technologies, Wilmington, Delaware) was used, consisting of a degasser, a binary pump, an injector/autosampler, a column heater, and a cooled thermostat controlled at 4°C autosampler compartment. Separation was performed using an Agilent Zorbax SB-C18, 4.6 $\times$ 150 mm (5 μ m) column equipped with a Phenomenex SecurityGuard analytical guard column. Both were maintained to 38°C throughout the entire analysis, eluted with a mobile phase of 50% v/v pure milliQ water with 0.1% v/v formic acid CovaChem (Sigma-Aldrich, St Louis, Missouri), and 50% v/v methanol Lichrosolv (Sigma-Aldrich, St Louis, Missouri). Bupivacaine was used as the IS (Sigma-Aldrich, St Louis, Missouri). The flow rate was set at 1 mL/min, and the injection volume was 10 μ L after needle washing with acetonitrile. The duration of each run was 4 minutes.

The chromatographic separation method was validated for 3 components of the dNCS, ie, lidocaine, mannitol, and sodium gluconate. Spiked calibrators and QC samples were

Table 2. Single Ion Monitoring Parameters.

Compound	Mass (Da)	Dwell time (ms)	Fragmentor (V)	Cell accelerator (V)	Polarity	Internal standard
Lidocaine	235.1	200	120	7	Positive	
Mannitol	205.1	200	110	7	Positive	
Sodium gluconate	219.0	200	120	7	Positive	
Bupivacaine	289.2	200	120	7	Positive	x

prepared with the closest possible matrix to the actual unknown samples rather than simple aqueous solutions. This ensured ionization patterns for the analytes of interest equal to those observed in the actual full formulation without possible artifacts and biased concentration readings.

With respect to lidocaine and mannitol, calibrators were obtained by spiking a dNCS solution prepared in the laboratory as per the pharmacy procedure, but just without the addition of those components in the formula. Such calibrators were freshly prepared on each assay day ranging in concentration at 8 levels from 0.03 µg/mL to 3 µg/mL, and 0.1 µg/mL to 6 µg/mL of lidocaine and mannitol, respectively. Sodium gluconate calibrators were prepared differently because of its presence in the Plasma-Lyte A 7.4 solution.

MS Acquisition Parameters

An Agilent 6410 Triple Quad mass spectrometry detector (Agilent Technologies, Wilmington, Delaware), with an electrospray ionization source, was used for detection and quantitation. Analysis was performed in positive mode with working parameters as follows: gas temperature 300°C; gas flow 11 L/min; nebulizer pressure 15 psi; and capillary voltage 4000 V. Quantitation was performed using the single ion monitoring mode as per the conditions listed in Table 2. Ion determination and qualitative information were obtained using scan mode with a range of 100 to 500 Da.

Results

The organoleptic analysis of each sample along the entire study revealed no precipitation, cloudiness, or color change in comparison with the original solution. The pH of the solutions under different storage conditions ranged between 6.5 and 7.1.

The LC-MS analytical method was precise and consistent. Calibration curves for all 3 analytes were linear with correlation coefficients averaging 0.9954 ± 0.002374 (mean $\pm$ standard deviation). Interday variability had a mean accuracy of 100.5% and a precision of 8.532% for sodium gluconate, 98.49% and 5.241% for lidocaine, and 99.96% and 6.747% for mannitol, respectively. A typical chromatogram is depicted in Figure 2. While lidocaine and the IS bupivacaine eluted separately with well-established retention times, mannitol and sodium gluconate co-eluted in single chromatographic peak. However, their individual quantification was achieved

by mass spectrometry given their distinct ionization ions and their respective masses, as shown in Figure 3.

With respect to lidocaine, its concentration averaged 0.117 mg/mL at 4°C for 30 days with a coefficient of variation of 4.2% over time, mainly attributable to sampling, handling, analytical and interbatch variability. This mean concentration represents 95.8% of the theoretical lidocaine titer of 0.122 mg/mL in dNCS. As shown in Figure 4, at 35°C the original concentration decayed in 30 days to about 67% of the original, while at 70°C nearly 50% of the titer was lost after the first day.

Fitting an exponential decay model to the data, the resulting semilogarithmic profiles were linear, in accordance to a first-order kinetics (Figure 5). The corresponding slopes and intercepts were estimated by linear regression to conduct an Arrhenius analysis (Figure 6).

According to the theoretical relationship between degradation first-order rate constants (k_{obs}) and the reciprocal of the absolute temperature (T),

$$\ln k_{\text{obs}} = \ln A - E_a / RT,$$

the activation energy (E_a) can be estimated from the slope, along with the Arrhenius factor (A) from the y -intercept, which represents the frequency of collision occurring on average between individual molecules (R stands for the ideal gas constant = 1.987 cal/oK.mol). From this analysis, an activation energy of 25 kcal/mol was estimated.

The exposure to light versus storage in the dark, both at a room temperature of 25°C, revealed no difference in the concentrations over time for neither of the analytes tested. This supports the assumption required by the Arrhenius analysis that degradation is only promoted by temperature.

The most stressed samples, held at 70°C under 80% relative humidity, were used to track the generation of degradation products. Comparing the mass spectra collected after 30 days of storage, all lidocaine, mannitol, and gluconate chromatographic peaks revealed ions not present at day zero and thus most certainly resulting from chemical degradation (Figures 7 and 8).

Discussion

The dNCS has become a major tool in cardiothoracic surgery in both pediatric and adult populations. Its use over the years

Figure 2. Typical chromatogram of a del Nido cardioplegia solution sample.

Note. IS = internal standard.

Figure 3. Qualifying ions for mannitol and gluconate.

Note. ESI = Electrospray ionization.

by many independent groups and institutions throughout the world has validated its intrinsic clinical value. This work intends to provide to the pharmaceutical and medical communities a definitive guidance for its production and storage. Because many hospitals and outsourcing

pharmacies are taking on the responsibility of compounding their own dNCS, this stability study also provides information about shelf-life and optimal storage conditions.

As far as the lidocaine component of dNCS, its intended concentration (0.122 mg/mL) is sustained up to 30 days

Figure 4. Lidocaine concentrations in del Nido cardioplegia solution, and moving averages trend lines over time at 3 storage temperatures.

Figure 5. Logarithmic decay of lidocaine concentrations over time at 3 storage temperatures.

Figure 6. Arrhenius plot.

under 4°C refrigeration. Likewise, the representative components sodium gluconate and mannitol revealed to be equally stable under the same storage conditions.

In accordance to current guidance,^{10,11} stability assays should be sufficiently specific to differentiate between the unaltered drug and any possible degradation products.

The issue is that many “strength” tests do not separate the intact drug from the degradation products, and the degradation products show up under one peak in the chromatogram, thus giving the false information that the drug concentration has not changed when it actually has. A stability-indicating assay, properly performed, will separate the degradation products/peaks and show the intact drug peak as it decreases in area or height, reflecting a change in the concentration of the intact drug [*sic*].¹²

Therefore, this study used stress conditions of temperature and humidity not so much to expect any extended stability, but rather to generate true positive spectra of degradation

Figure 7. Degradation products of lidocaine peak at 4°C 30 days (A) and at 70°C 30 days (B).

Figure 8. Degradation products of mannitol/gluconate peak at 4°C 30 days (A) and at 70°C 30 days (B).

products. We succeeded in generating a number of such degradation products which were screened for in the original and stable refrigerated samples. The absence of such degradation products in those samples corroborates the stability of the

dNCS solution under the most updated criteria defined by the Food and Drug Administration and the USP. Not only are the titers of its major components unaltered, but there are also no confounding degradation products.

Based on both physical appearance and chemical composition, extemporaneously prepared dNCS can be stored for up to 30 days under refrigeration at 4°C in EVA bags for standard usage.

Our institution currently requires commercially prepared dNCS to be utilized, as per recent state legislation (ie, Pharmacy Practice Act, Commonwealth of Massachusetts, House Bill, No. 3672, amended, accompanying bill (House, No. 4235), June 29, 2014). Whenever in-house preparation becomes required again, we will modify our dNCS labels for a 30-day expiration date when kept refrigerated at 4°C.

Conclusions

The dNCS is stable for at least 30 days under 4°C refrigeration. No degradation products are generated during this storage time in EVA bags under these conditions for the components measured.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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