Fresenius Medical Care–North America has developed a neutral-pH version of its Delflex peritoneal dialysis (PD) solution with low glucose degradation products (GDPs): Delflex Neutral pH. The Delflex Neutral pH system stores the PD solution in a dual-chamber bag. The product is admixed by the patient before use. The new design facilitates GDP reduction in two ways. First, GDPs are reduced because the dextrose solution is stored at a pH that minimizes degradation during sterilization and that optimizes dextrose stability over time. Second, the design minimizes generation of acetaldehyde by separating dextrose from lactate during heat sterilization of the product. Mixing the contents of the two chambers before use produces a physiologically compatible pH of approximately 7.0, with minimal GDPs.

Analysis of GDP content was conducted by high-performance liquid chromatography. The GDP reduction across all sizes and formulations of Delflex Neutral pH ranged between 74% and 93% as compared with conventional Delflex PD solution. Testing of the new delivery system by prevalent PD patients demonstrated that, with minimal training, patients can obtain a homogeneous PD solution low in GDPs with a physiologically compatible pH of approximately 7.0.

Introduction

Conventional peritoneal dialysis (PD) fluids are acidic and contain high levels of glucose degradation products (GDPs) as a result of the heat sterilization process. During sterilization and extended storage periods, dextrose degrades into GDPs, which are potentially harmful molecules. The dextrose molecule naturally interconverts between an annular and a linear structure. In aqueous solution, the linear dextrose molecule exists in equilibrium with 1,2-enediol, which is susceptible to degradation reactions.

Under elevated temperatures and in mildly acidic conditions (the pH range typical of conventional PD solutions), 1,2-enediol will form 3-deoxyaldose-2-ene, which in turn forms the GDPs 3-deoxyglucosone (3-DG) (1,2) and 3,4-dideoxyglucosone-3-ene (3,4-DGE) (3). The 3,4-DGE will then decompose into 5-hydroxymethylfurfural (5-HMF). The GDPs, glyoxal (Glx), methylglyoxal (M-Glx), and furaldehyde stem from 1,2-enediol and 3-deoxyxylulose-2-ene decomposition. Furthermore, dextrose will also react with lactate (present in most PD fluids as an osmotic agent and pH buffer) to form acetaldehyde (AcA) during heat sterilization (4).

By storing the dextrose component of Delflex Neutral pH PD fluid (Fresenius Medical Care–North America, Waltham, MA, U.S.A.) at a pH below 4.0, the reactions that lead to GDP formation are substantially reduced (5). By placing the buffer solution (a mixture of lactate and bicarbonate) in a compartment separate from the dextrose, the GDP AcA is almost entirely eliminated (6–8).

The mixing of the two components, an acidic dextrose solution and a basic lactate/bicarbonate solution results in a low-GDP solution with a physiologic pH of approximately 7.0.
The aim of the research reported here was two-fold: First, quantify the reduction of seven GDPs in the new bicarbonate/lactate–buffered Delflex Neutral pH PD solution as compared with standard Delflex PD solution (Fresenius Medical Care). Second, evaluate the effectiveness of a new two-compartment PD container closure system and assess how well patients are able to mix the two-component product to produce a homogenous PD fluid.

The Delflex Neutral pH product was also tested in a simulated-use study by current automated PD (APD) and continuous ambulatory PD (CAPD) patients to determine how well patients could manipulate the new two-compartment PD container, mix the PD solution, and dispense a homogenous PD fluid. Patients dispersed the product into an artificial peritoneum, where pH measurements of the mixed PD fluid were used to assess homogeneity.

Materials and methods

Samples for analysis
Conventional Delflex and Delflex Neutral pH PD solutions are manufactured at dextrose concentrations of 1.5%, 2.5%, and 4.25%. Product fill volume, air volume within the overwrap plastic, and sterilization parameters affect final GDP levels. All product consumed in the present study was manufactured at nominal parameters (fluid fill, air in overwrap, and sterilization cycle). Conventional Delflex PD solutions served as control samples and were obtained from normal production runs. All PD fluid used in the current study was analyzed for GDPs within 90 days of production.

Analysis of GDPs
Before high-performance liquid chromatography (HPLC) analysis, all PD solution samples were derivitized with either o-phenylenediamine (OPD) when analyzing for 3-DG, Glx, and M-Glx, or with dinitrophenylhydrazine (DNPH) when analyzing for 5-HMF and the aldehydes. Following derivitization, all samples were loaded into vials and placed on the HPLC autosampler. Analyses of GDPs in conventional PD fluids and in Delflex Neutral pH solution were conducted using an Agilent 1100 Series ChemStation for HPLC (Agilent, Santa Clara, CA, U.S.A.) equipped with an Agilent multiple wavelength detector, model G1365B.

In the case of 3-DG, Glx, and M-Glx, PD samples were derivitized by combining 100 μL OPD with 100 μL PD fluid and by adding 10 μL 1.0 mmol/L 2,3-butanedione, 160 μL 2 mmol/L HClO₄, and 80 μL 1% OPD. Samples were then vortexed for 3 seconds and incubated at 25°C for 3.0 hours. A Phenomenex Gemini 5μ C18 5μm×4.6 mmol/L×50 mmol/L column (Phenomenex, Torrance, CA, U.S.A.) was then used to separate 3-DG, Glx, and M-Glx with gradient elution [A: 0.10% trifluoroacetic acid (TFA) in H₂O; B: 0.08% TFA in a mixture of 80% H₂O and 20% acetonitrile (4,9)].

In the case of 5-HMF and the aldehydes, 500 mL PD fluid was combined with 100 mL DNPH solution. The DNPH solution was prepared from 0.05 g DNPH, 200 μL sulfuric acid, 300 μL H₂O, and 9.5 mL acetonitrile. Samples were mixed and allowed to incubate at 25.0°C for 1.0 hour and were then spiked with 900 μL acetonitrile. After derivitization, 5-HMF and aldehydes were separated using a Phenomenex Luna 5μ C18 3μm×4.6 mmol/L×50 mmol/L column with a flow rate of 1 mL/min and gradient elution (A: methanol; B: phosphate buffer solution).

Simulated use by study participants
A simulated-use study involving actual Delflex PD patients was conducted to assess product usability and product performance. The patient pool for the study consisted of 24 patients (10 women, 14 men; age range: 18 – 78 years) who were chosen from three different clinics located within the continental United States. Participants were current home APD or CAPD patients using Fresenius Stay•Safe brand solution.

On the day that they performed the simulated-use test, participating patients were trained at their local clinic in how to mix and use the two-compartment PD fluid. Patients infused the Delflex Neutral pH PD fluid into a mock peritoneal cavity for pH sampling purposes. Training consisted of a demonstration by a PD nurse with the actual product, reading of the “instructions for use” included with each product case, and referral to a procedural card with step-by-step photos. Each patient completed four independent uses of the product.

Outflow pH sampling
Measurements of the pH of the infused Delflex Neutral PD solution were used to determine product homogeneity after mixing. The pH was sampled by
withdrawing an aliquot (200 mL) of PD fluid at 3 stages—0%, 45%, and 90% dispensed volume—during a mock infusion process. These sampling points were selected to observe product pH at stages at which deviations from the nominal pH were most likely to occur. Aliquots were withdrawn from the transfer line at the specified intervals and dispensed into sealed containers for pH measurement.

The pH measurements were made using a SevenMulti pH meter (Mettler Toledo, Columbus, OH, U.S.A.) equipped with a pH/mV expansion module and a pH probe (InLab Routine Pro: Mettler Toledo) with automatic temperature compensation. The pH probes were calibrated using high-precision pH buffers (pH 4.000, 7.000, and 10.000) obtained from Ricca Chemical (Pequannock, NJ, U.S.A.).

Results

GDPs
Table I summarizes the concentrations of seven individual GDPs detected in the two solutions. The GDP (3-DG, 5-HMF, M-Glx, Glx, AcA, formaldehyde, and furaldehyde) concentrations are reported in micromoles per liter. Analyte concentrations below the detection limit are listed as not detected (ND). Results are the average of 9 samples.

Outflow pH data
Three dialysate samples (at 0%, 45%, and 90% of solution dispensed) were collected from each bag mixed during the simulated use study. Of the three samples collected from each simulated use, the first sample showed the widest range in measured pH. Figure 1 shows the pH results from the first pH dataset (0% dispensed), which has a normal distribution and a mean value of 6.84 (95% confidence interval: 6.74 to 6.93). All subsequent pH datasets (at 45% and 90% dispensed) had narrower distributions and similar medians (data not shown).

To try to understand how patient mixing errors could potentially affect the pH of the dispensed PD fluid, Figure 2 shows overlay histograms of two data subsets from the first pH dataset. Subset 1 (n = 60) consists of samples from bags processed by patients without mixing errors. Subset 2 (n = 13) comprises samples from bags for which patients committed major mixing errors that could potentially affect the final product pH. The pH data from simulated uses in which patients made minor mixing errors were not included in the comparison.

Both datasets were normally distributed (confirmed by a Shapiro–Wilks W-test, at a confidence level of 95%). The mean values of the “no-error” and “error” datasets were 6.84 and 6.84, with 95% confidence intervals of 6.73 to 6.94 and 6.75 to 6.92 respectively. A two-sample t-test for difference of means concluded that the means were not statistically significantly different, at a 95% confidence level. The “error” pH data 95% confidence interval falls within the “no error” confidence interval, and both 95% confidence intervals fall within the pH specification range for the product.

Discussion
In Delflex Neutral pH solution (as compared with conventional Delflex PD solution), we observed a
total reduction of GDP levels by 74% – 93%. Compared with conventional Delflex solution, Delflex Neutral pH contained at least 50% less 3-DG and almost no AcA.

For pH measurement, dataset 1 is the most likely to reveal deviations from the optimal dispensed pH. All first pH measurements in the simulated use study ranged between 6.70 and 7.05. The mean pH for the product obtained during release testing at our quality control laboratory was 6.820. Good agreement between the first pH measurement, the middle pH measurement (not shown), and the last pH measurement (not shown) demonstrated that the product was homogenous after completion of the mixing steps, regardless of dispensed volume.

A comparison between the pH measurements in dataset 1 for simulated use with no errors and simulated use in which patients made mixing errors showed that, despite mixing errors, the two subsets of pH data cannot be statistically distinguished.

Conclusions
The simulated-use study demonstrated that prevalent PD patients could learn the new system in under 2 hours and that the new Delflex Neutral pH system consistently delivered a PD fluid with a pH of approximately 7.0.

References

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