SHORT REPORTS

Cephazolin and Gentamicin Are Stable in Lactate-Buffered Fresenius Peritoneal Dialysate for Seven Days at Room Temperature

Peritoneal dialysis (PD) peritonitis is a common complication of PD and is associated with significant morbidity and mortality (1). Treatment involves intraperitoneal delivery of antibiotics, which, for this condition, is superior to the intravenous route (2). Outpatient treatment involves loading antibiotics into bags of dialysate, which are then self-administered. However, some patients are unable to load the antibiotics themselves and therefore rely on pre-loaded bags provided to them by health care staff. Treatment for PD peritonitis is usually required for no less than 14 days, and it is desirable to pre-load at least several days' worth of bags for convenient outpatient therapy (3). That pre-loading in turn depends on the stability of the required antibiotic in solution under conditions of home storage. However, relevant data on antibiotic stability is limited to just some of the available PD solutions and containers (4–7). Guidelines, such as those published by the International Society for Peritoneal Dialysis provide information about the stability and compatibility of various antibiotics in peritoneal dialysate, although they note the limited data for specific PD solutions and suggest the need for further study (3). It has also been suggested by others that data obtained from experiments in one solution or system cannot be extrapolated to another (4).

A commonly used PD system is the polyvinyl chloride twin-bagged lactate-buffered glucose-based solution from Fresenius (ANDY-Plus/Disc: Fresenius Medical Care, Bad Homburg, Germany). The stability of antibiotics in this system has not been widely studied and cannot be confirmed by the manufacturer. The aim of the present study was therefore to assess the stability of cephazolin and gentamicin in that dialysate under conditions that simulate up to 7 days of home storage, to ensure effective and convenient use for outpatient treatment of PD peritonitis.

METHODS

We investigated the antibiotics at concentrations and combinations typically used during guideline-based treatment of PD peritonitis: either cephazolin 2 g (DBL Cephazolin Sodium: Hospira, Lake Forest, IL, USA) or gentamicin 60 mg (Pfizer Gentamicin: Pfizer Pharmaceuticals, New York, NY, USA), or both, added to 2-L bags of 2.3% dextrose dialysate in standard clinical fashion using sterile technique.

For each antibiotic or combination, we prepared 3 pre-loaded bags and incubated each bag at 18°C – 21°C, out of direct sunlight, in 50% ± 10% humidity (a typical value for an indoor environment) for various periods of time: 1 bag for 7 days, 1 bag for 3 days, and 1 bag for an hour. The experiment was performed in triplicate for each antibiotic or combination and for each incubation period (Figure 1). At the end of incubation, to replicate usual clinical practice, we used heating plates to heat the bags to 35°C over 40 minutes. We then used sterile technique to sample aliquots from each bag and submitted those specimens immediately for analysis.

Sample Analysis: We measured the responses of the antibiotics in the samples by liquid chromatography–mass spectrometry (LC/MS) on a QStar XL Hybrid quadrupole time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA, USA). All samples were analyzed on the same day to minimize assay variability.

The LC/MS method was chosen over the high-performance liquid chromatography (HPLC) method because of its superior selectivity in discriminating a drug from its degradation products. Although both methods benefit from LC separation, simple ultraviolet traces from HPLC analyses will not reliably demonstrate the true composition of a drug peak if its degradation products share the same retention time and have an absorbance range that at least partially overlaps the range being monitored for the drug. The use of high-resolution MS allows for selective monitoring of narrow mass ranges that correspond to the structures in question, which will be highly specific under the reasonable and customary assumption that changes in chemical structure through degradation will result in a change

Figure 1 — Schematic of the experimental design. LC/MS = liquid chromatography–mass spectrometry.

in molecular weight, moving such species out of the monitored range. It is possible to envisage a highly unlikely theoretical scenario in which a degradation product not only shares the same retention time as the drug, but also has an isotopic profile that overlaps with the narrow mass range being monitored; however, in almost all cases, the LC/MS methodology will still outperform a standard HPLC method when it comes to discriminatory power.

Gentamicin is a mixture of 4 different molecules with 3 different molecular weights, all of which had to be monitored in the present study. We developed a binary mobile phase system using 0.1% formic acid and 0.01% heptafluorobutyric acid in water (buffer A) and in acetonitrile (buffer B). A solvent gradient was developed such that the 4 components of gentamicin were resolved on a capillary C18 column. Two internal standards were spiked into every sample to account for possible variations in instrument response during the course of the analyses. Kanamycin nearly co-eluted with gentamicin under the established conditions and so was chosen as the internal standard for that analyte. Cephazolin eluted far later than gentamicin, and so to correct for any deviations in signal strength at this retention time, vancomycin was spiked in. The observed mass-to-charge

ratios were 455 for cephazolin, 485 for kanamycin, and 724.7 for vancomycin (2+ charge state). The forms of gentamicin generated distinct extracted ion chromatograms, with mass-to-charge ratios of 450, 464, and 478 for the various positively-charged species. The trace for mass-to-charge 464 represents the combined signal for the two structural isoforms with identical atomic composition, which co-eluted in our LC separation. Because the LC/MS analysis could clearly differentiate between the gentamicin forms and cephazolin, we pooled the single-drug bags for LC/MS analysis.

To produce a metric that would be more robust than a simple “area under the curve” for the drugs, we derived an analyte area ratio by dividing the analyte peak area by the internal standard peak area. Those ratios were used to calculate change over time. The coefficient of variation for the assay was less than 15%, meeting the Food and Drug Administration standard for an analytical method of 20% or lower (8).

Finally, although we were not able, because of budget constraints, to perform extensive or definitive methods validation, we believe that we have established satisfactory sensitivity and precision and also that the inherent specificity of LC/MS (compared with HPLC, for example) provided plausible and credible measurements.

Statistical Methods: We used regression models to analyze our data as a cross-sectional time series. Two statistical models were developed, one for all analytes together and one for each analyte separately. In the combined-drug model, analytes were specified as random effects, assuming different random effects for different analytes, with coefficients estimated using maximum likelihood. In the individual-drug model, analytes were specified as fixed effects. Time was modeled as a continuous variable. The generalized form of the equations used is

$$\gamma = X\beta + Z\mu + \varepsilon,$$

where γ is the $n \times 1$ vector of responses, X is an $n \times p$ design or covariate matrix for the fixed-effects β , and Z is the $n \times q$ design or covariate matrix for the random-effects μ . Observed results are expressed as antibiotic mass (analyte area ratio), and modeled results are expressed as percentage change in antibiotic mass.

We used Stata Intercooled 12 (StataCorp LP, College Station, TX, USA) for the analysis, and a p value less than 0.05 was considered statistically significant.

RESULTS

Figure 2 shows the observed mass of the antibiotics cephazolin and gentamicin in the samples at various time points, expressed as the percentage of the mass observed at each time point relative to the mass at 1 hour. Customarily, antibiotic stability is defined as 90% of the initial concentration remaining (4), and that threshold is indicated on our graphs by dotted lines.

With the antibiotics modeled altogether, we found a nonsignificant decline in overall antibiotic mass of -0.143% per day ($p = 0.759$). With the antibiotics modeled separately, we found a statistically significant decline in the mass of two of the gentamicin molecules: a change of -1.598% per day for gentamicin 450 ($p = 0.011$) and -1.26% per day for gentamicin 478 ($p = 0.039$). Those declines were seen only in gentamicin-loaded bags and not in bags loaded with both gentamicin and cephazolin. Changes in the mass of other antibiotics were not statistically significant (Table 1).

We observed no physical signs of incompatibility—such as sedimentation or clouding—in any of the samples.

DISCUSSION

Despite similar glucose concentrations, peritoneal dialysate from different manufacturers can vary considerably with respect to pH, other fluid components, and characteristics of the storage container.

Extrapolation of antibiotic stability data from one PD system to another is therefore invalid, and ongoing study to monitor antibiotic stability in various systems is necessary (4).

The present study demonstrates satisfactory stability of cephazolin and gentamicin in Fresenius ANDY-Plus/Disc peritoneal dialysate, under conditions that simulate home storage for 7 days. We observed no change in the detectable mass of cephazolin in the dialysate over that period. We did observe a subtle decline in the mass of two of the four component molecules of gentamicin, but only at a rate of less than 1.6% per day, which is unlikely to be of clinical significance. Our results support findings from previous studies of these drugs in various PD fluid systems, which have reported acceptable stability alone and in combination for up to 8 days when stored at less than 25°C , but for a much shorter period at higher temperatures (5,7,9–11).

Notably, the observed decline in the mass of gentamicin was not seen in bags containing gentamicin in combination with cephazolin. We can speculate on several potential explanations for that finding, although none can be determined from the study. First, the small declines in mass observed with gentamicin ($\pm$ cephazolin) might lie within, or close to, the error of the method. However, if we accept the finding as real, it could potentially be attributable to cephazolin preventing the adsorption of gentamicin onto bag surfaces, or the effect of cephazolin temporarily stabilizing gentamicin. Finally, it is possible that the finding is an artifact of batch variability in the proportion of the types of gentamicin in the proprietary product used for the study. With respect to that product, its final composition is within the limits specified in the British Pharmacopoeia: gentamicin 450 ($\text{C}_{19}\text{H}_{39}\text{N}_5\text{O}_7$) at $10\% - 35\%$, gentamicin 464 ($\text{C}_{20}\text{H}_{41}\text{N}_5\text{O}_7$) at $25\% - 55\%$, and gentamicin 478 ($\text{C}_{21}\text{H}_{43}\text{N}_5\text{O}_7$) at $25\% - 50\%$ (12). However, no information is available from the manufacturer on batch variability. Some variability might be therefore expected, but perhaps to a lesser degree than is observed between products from different manufacturers.

The option of pre-loading antibiotics into peritoneal dialysate is an essential tool for the treatment for PD peritonitis in patients who cannot themselves load antibiotics into dialysate. Our study indicates that pre-loading of antibiotics into Fresenius ANDY-Plus/Disc dialysate for a week is not likely to compromise treatment efficacy, potentially allowing for weekly preparation of pre-loaded PD bags and delivery to patients, and thus inexpensive and efficacious treatment in an optimal outpatient environment.

Figure 2 — Observed mass of antibiotics at various time points (medians with interquartile error bars). Mass is expressed as the percentage of the mass observed at each time point relative to the mass at 1 hour. The threshold for antibiotic stability (90% of the initial concentration remaining) is indicated by dotted lines.

The practicality of the pre-loading approach is limited by two factors. First, once sensitivities are obtained from the initial PD culture, antibiotic use is often more

specifically targeted. In many cases, the prescription for intraperitoneal antibiotics will change after 48 hours. Second, there is the issue of bacterial contamination.

TABLE 1
Change in Observed Mass of Antibiotics
Modeled Over Time

Analyte	Modeled change per day (%)	<i>p</i> Value
All	−0.143	0.759
Cephazolin	0.402	0.404
Cephazolin (+ gentamicin)	−0.429	0.606
Gentamicin 450	−1.598	0.011 ^a
Gentamicin 450 (+ cephazolin)	−0.975	0.156
Gentamicin 464	−0.881	0.116
Gentamicin 464 (+cephazolin)	0.348	0.715
Gentamicin 478	−1.26	0.039 ^a
Gentamicin 478 (+cephazolin)	1.0268	0.492

^a *p* < 0.05.

Contamination was not measured in the present study, and it could potentially be a problem with a longer incubation period for the (albeit antibiotic-containing) peritoneal dialysate. For that reason, we suggest that any change to weekly preparation and delivery of pre-loaded PD bags be grounded in a formal quality improvement initiative to ensure clinical effectiveness. If bacterial contamination were to be identified as an issue, the requirement for antibiotics to be pre-loaded by a pharmacist in a sterile dispensing suite might offset many of the aforementioned logistic benefits. Notwithstanding, we believe weekly preparation and delivery of pre-loaded PD bags to be a likely option for improving health care delivery for most patients with PD peritonitis.

It is important to note that this was a quantitative study assessing detectable antibiotic mass; functional antimicrobial activity of the stored peritoneal dialysate was not assessed. In practice, assessing the stability of antibiotics by measurement of antimicrobial activity is subject to wide variability and might not reliably reflect antibiotic stability (4,5). In addition, because of the high concentration of antibiotics in peritoneal dialysate in this setting, antimicrobial activity is likely to be less sensitive to the relatively smaller changes in antibiotic concentration observed in the present study.

SUMMARY

We found cephazolin and gentamicin, either alone or in combination, to be stable in Fresenius ANDY-Plus/Disc 2.3% peritoneal dialysate for up to 7 days when stored at room temperature, thus allowing for pre-loading of antibiotics into PD bags to facilitate outpatient treatment of PD peritonitis episodes.

DISCLOSURES

The study was supported by the Counties Manukau District Health Board. The authors have no financial conflicts to declare.

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