Antibiotic stability in commercial peritoneal dialysis solutions: influence of formulation, storage and duration

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Abstract

Background. Peritoneal dialysis (PD)-associated peritonitis is treated by administration of antibiotics mixed with the PD solution. Data on antibiotic stability for solutions in current use are limited. The aim of this study was to determine the stability of cefepime, cephazolin and ampicillin in three commercial PD solutions.

Methods. Antibiotics were added to the non-glucose compartment of the Gambro (Gambrosol®/C210) and Fresenius (Balance®/C210) multi-compartment systems and Baxter (Dianeal®) single-compartment (glucose 2.5%) PD solutions in a sterile suite. Antibiotic stability over 3 weeks was determined using both a bioassay of bacterial inhibition and antibiotic concentrations. The influence on stability and sterility of storage conditions was determined.

Results. The bioassay demonstrated the stability of all antibiotics for 9 days at room temperature and 3 weeks when refrigerated, except ampicillin in the Gambro solution, which displayed no bioactivity after 4 days. However, a ceiling effect in bacterial inhibition at higher antibiotic concentrations limited the ability of the bioassay to detect antibiotic degradation at relevant concentrations. Antibiotic concentrations varied with time but were comparable to the bioassay and supported stability in refrigerated solutions, except ampicillin in the Gambro solution. No bacterial contamination, marked colour change or precipitation occurred.

Conclusions. This study supports the stability of cephazolin and cefepime in all three PD solutions and ampicillin in only the Baxter and Fresenius PD solutions. Antibiotic stability studies should ideally be conducted prior to registration and marketing of new PD solutions.

Keywords: antibiotic stability; intraperitoneal antibiotics; peritoneal dialysis; peritonitis

Introduction

Peritoneal dialysis (PD) is a widely used form of renal replacement therapy for patients with end-stage renal failure, particularly in developing countries because it is relatively cheap and convenient compared to haemodialysis [1]. PD-associated peritonitis (PD-peritonitis) is a complication of PD that causes pain, systemic illness and often requires hospital admission. In Australia, it develops in 69% of patients within the first 3 years of treatment and is the second-most common reason for patients ceasing PD [2]. Appropriate management of PD-peritonitis may prevent or delay conversion from PD to haemodialysis, which has important implications for the patient (quality of life) and health system (decreased costs).

Best practice management of PD-peritonitis is intraperitoneal (i.p.) administration of antibiotics. This is superior to intravenous therapy [3] and allows ongoing outpatient treatment, which shortens hospital admission with benefits to the patient and the health system. Antibiotics are administered (i.p.) for up to 3 weeks following an episode of PD-peritonitis. To be suitable for home-based patient self-administration, the antibiotic should be stable in the PD
solution for a number of days under home storage conditions. This is particularly important for patients who live in regional areas where frequent travel to the local tertiary hospital is difficult. Some centres train patients to add antibiotics to their own PD solutions immediately prior to administration; however, this method is only appropriate for patients with the skills and ability to adhere to aseptic techniques.

A range of antibiotics are utilized worldwide for the treatment of PD-peritonitis [3, 4]. Antibiotic choice is best determined using local data on the basis of common infecting organisms and their antimicrobial sensitivity. In general, cephazolin and gentamicin are administered first-line, unless microbiology isolate data identify a resistant organism, in which case second-line agents such as cefepime and vancomycin are often used. In Australia, vancomycin is administered to ~50% of patients with PD-peritonitis [2, 4] and to 94% of cases where Enterococcus is grown, contrary to international guidelines (that recommend ampicillin) [5]. Where possible, vancomycin use should be restricted to limit the development of multi-resistant organisms. Underuse of ampicillin in Australia may relate in part to limited stability data, yet ampicillin with sulbactam is used in other units worldwide given its improved efficacy against Enterococcus and anaerobes [6–8], despite the absence of published stability data.

There are limited data regarding current commercial formulations and duration of antibiotic activity for some of the antibiotics listed above. Plasma drug concentrations of gentamicin and vancomycin are readily determined to confirm exposure, but therapeutic drug monitoring of other antibiotics used in the treatment of PD-peritonitis such as cephazolin, cefepime and ampicillin are not widely available. Therefore, in vitro studies to quantify antibiotic activity in PD solutions are necessary to maximize the effect of treatment.

Such studies should be conducted in currently available commercial PD solutions because antibiotic activity may either decrease or increase in PD solution compared with saline. PD solutions in Australia are sold by three companies: Fresenius, Baxter and Gambro. They were initially marketed in varying glucose concentrations (single-compartment PD solutions) and stability studies were conducted on these products at the same time points as those listed for changes in pH, colour and particulate matter. Bioassays were conducted on 2–3 different days, with two to three replicates on each day.

In vitro antibiotic stability data from one formulation does not necessarily apply to other formulations [9]. Therefore, studies assessing antibiotic activity in a variety of storage conditions over a prolonged period of time are required.

The aims of this study are to determine the stability of commonly used antibiotics in PD solutions and to understand the impact of physical composition of PD solutions on antibiotic compatibility.

Materials and methods

The antibiotics and concentrations were selected according to the international guidelines: cephazolin 500 mg/L, cefepime 500 mg/L and ampicillin 500 mg/L. Changes in stability and antibiotic activity were determined in three PD solutions: Gambro multi-compartment system (101921—Gambrosol Tri-040820N), Fresenius multi-compartment system (AP29029—CAPD 19 balance 2.5%, 2500 mL) and Baxter single-compartment system (79473—fluid Dianeal 2.5% glucose, 1.25 mmol calcium, 3 L).

Commercial brands of antibiotics registered for intravenous use in Australian Hospitals were used: Austrapan, Lennon Healthcare (ampicillin), DBL Cephazolin, Hospira and Maxipine, Bristol–Myers Squibb (cefepime). Antibiotic bags were reconstituted in the pharmacy sterile dispensing area by experienced staff in the pharmacy sterile dispensing unit at The Canberra Hospital. The antibiotic was reconstituted using sterile water according to the manufacturer instructions. The protective packaging of the PD solution was opened, the antibiotic was admixed by injecting the reconstituted solution into the injection port, and this was then covered with a foil seal. The PD bag was then returned to a protective packaging and resealed until sampling. Five PD solutions were prepared for each antibiotic in each PD solution to test various conditions, as follows:

(a) Storage in refrigeration (4°C), with sampling on Days 0, 2, 4, 6 and 9 to measure bioactivity and antibiotic concentration.
(b) Storage at room temperature (25°C), with sampling on Days 0, 2, 4, 6 and 9 to measure bioactivity and antibiotic concentration.
(c) Storage in refrigeration (4°C) for 7 days, then a single sample was taken to assess sterility, bioactivity and antibiotic concentration.
(d) Storage in refrigeration (4°C) for 14 days, then a single sample was taken to assess sterility, bioactivity and antibiotic concentration.
(e) Storage in refrigeration (4°C) for 21 days, then a single sample was taken to assess sterility, bioactivity and antibiotic concentration.

All samples were obtained using sterile technique from the injection port after each bag had been thoroughly mixed. The sample on Day 0 was taken within hours of addition of the antibiotic. At each time point, 5 mL of PD solution was removed to determine the bioactivity of the solution and antibiotic concentration as a measure of antibiotic stability and compatibility over time. Previous studies have shown that bioactivity decreases to a greater extent than antibiotic concentration [10] so these were assessed according to the latest guidelines [9]. Bioactivity reflects both stability and compatibility of the antibiotic with the dialysate fluid rather than just the concentration (which, depending on the type of assay, may include inactive but measurable antibiotic).

Samples for quantification of the drug concentration were immediately stored at –80°C until being transported on dry ice to Queensland Health Pathology Service at Royal Brisbane and Women’s Hospital, Queensland, Australia. The concentration of cephazolin, ampicillin and cefepime were determined by high-performance liquid chromatography (HPLC). The coefficient of variation was generally <5% and the limit of quantification was 1 mg/L [11]. Samples were defrosted and analysed on the same day. To estimate the extent of spontaneous hydrolysis with the freeze-thaw cycle, the samples were reanalysed 3 months later.

Bioactivity is the extent to which the antibiotic-dialysate solution inhibits bacterial growth. The protocol was based on a previously described method [3]. Bacterial control strains were used for each antibiotic as follows: Pseudomonas aeruginosa ATCC 27853 for cefepime, Enterococcus faecalis ATCC 29212 for ampicillin and Staphylococcus aureus ATCC 25923 for cephazolin. A 0.5 McFarland density of the appropriate organism was prepared in Mueller–Hinton (MH) broth and evenly inoculated onto an MH agar plate. Twenty microlitres of dialysate was added to a blank Kirby–Bauer disc, which was then applied to the relevant MH agar plate. The plates were incubated in air at 35°C for 16–24 h. Inhibitory zone diameters were measured manually. Changes in the zone of inhibition relative to time were described descriptively.

Standard curves demonstrating the relationship between antibiotic concentration and bioactivity were constructed. The above methodology utilized antibiotic solutions following serial dilution with sterile water to the following concentrations: 1000, 500, 250, 125, 62.5 and 0 mg/L (sterile water), for ampicillin 31.25 mg/L was also prepared. These were conducted on 2–3 different days, with two to three replicates on each day.

Loss of sterility of the PD fluid due to contamination was assessed using the Bactec system. Eight millilitres of PD fluid was inoculated into aerobic and anaerobic Bactec blood culture bottles and incubated for 7 days as per standard protocol for blood cultures.

To control for the influence on storage and to assess for intrinsic bioactivity of the PD fluids, one bag of each PD solution was stored under the same conditions as (b)–(e), without admixed antibiotics, and assessed for changes in pH, colour and particulate matter. Bioassays were also conducted on these solutions at the same time points as those listed above.

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Results

The bioactivity standard curves are shown in Figure 1. The relationship between the zone of inhibition and antibiotic concentration was non-linear, particularly at high concentrations where the incremental increase in inhibitory diameter was relatively small. The variability in bacterial inhibition appeared minimal, suggesting good reproducibility by this assay.

Changes in bioactivity in the PD solutions with admixed antibiotics are shown in Figure 2 for cephazolin, cefepime and ampicillin. Results from PD solutions (c), (d) and (e) were incorporated with those of (a) to show the effect of prolonged storage by refrigeration on antibiotic activity. On visual inspection of these bioactivity graphs, with the exception of ampicillin in the Gambro formulation (Figure 2), the inhibitory diameter did not change markedly over the 3 weeks. By comparison of these inhibitory diameters to the bioactivity standard curves (Figure 1), the expected concentration of cephazolin is \( \geq 125 \text{ mg/L} \) in the Baxter solution and \( \geq 250 \text{ mg/L} \) in the Fresenius and Gambro solutions. Similarly, in the case of cefepime, the inhibitory diameters are consistent with a concentration \( \geq 250 \text{ mg/L} \) in each PD solution. For ampicillin, the concentration remained \( \geq 125 \text{ mg/L} \) in the Baxter solution and \( \geq 250 \text{ mg/L} \) in the Fresenius solution, but for the Gambro formulation, there was minimal bioactivity after 4 days (Figure 2).

Changes in antibiotic concentration in the PD solutions are shown for cephazolin, cefepime and ampicillin in Figure 3. The concentrations fluctuate, despite the sample being obtained in some cases from the same PD solution. Overall, the concentrations measured by drug assay were higher than those predicted by bioassay. In the case of ampicillin, no antibiotic was detected in the Gambro PD solution in contrast to the results for bioactivity (Figure 2) where some ampicillin was present for the first 4 days. There was a trend towards lower concentrations in the PD solutions stored at room temperature compared to those stored in refrigeration. Reanalysis of the samples 3 months later demonstrated an \(-15\%\) decrease in concentration compared to the initial result.

The pH of the Baxter PD solutions was 5 at each time point regardless of the admixed antibiotic. The pH of the Fresenius solution was 8 for all solutions except ampicillin, which was initially 8 but increased to 9 from Day 4. The pH of the Gambro solution increased from 5 to 6 on Day 4 for those with admixed cefepime and cephazolin. In contrast, the pH of Gambro solution containing ampicillin was 7–8. A faint yellow colour change was noted in the solutions with admixed cefepime from Day 0. No sediment or clouding of the PD solutions was noted. There was no evidence of bacterial contamination in any sample.

Discussion

To our knowledge, this is the first study measuring the stability of cephazolin, cefepime and ampicillin in three commonly used PD solutions in Australia over a prolonged period. With the exception of ampicillin in the Gambro PD solution, we have demonstrated acceptable compatibility and stability for all antibiotics stored in refrigeration. This information supports the practice of sterile admixing of cephazolin and cefepime to a number of PD bags for use over days to weeks.

Antibiotic stability is essential for the efficacy of i.p. administration of antibiotics. In vitro stability data from one formulation do not necessarily apply to other formulations [9]. Variations in the formulation of PD solutions, including the concentration of glucose, influence compatibility and stability of admixed antibiotics [9, 12]. Stability is determined by degradation of the drug in solution (e.g. hydrolysis), which is influenced by temperature and humidity and should conventionally be <10%, for a drug to be considered ‘stable’. Incompatibility is a loss of antibiotic activity due to interactions with the PD formulation, including adsorption to the container [9].

An extensive review of the literature and communication with product representatives confirmed that there are no current data on the stability of antibiotics in the new PD solutions. As noted previously, more research on drug stability in PD solutions is required [4, 9]. It is suggested that antibiotic stability and compatibility are measured using both direct quantification of the antibiotic concentration and indirectly using a bioassay [9]. In our study, the plateau in bacterial inhibition with increasing antibiotic concentrations limits the ability of the standard curves to discriminate between relatively large changes in concentration (e.g. a decrease from 1000 to 500 mg/L) using bioactivity data only. This also relates to the control bacteria used in the bioassay, where selection of bacteria with a higher minimum inhibitory concentration may limit this plateau effect. As such, it is useful to compare these results to a direct drug assay.

Quantification of the concentration of antibiotics using a specific assay like the one used in our study is more useful in stability testing because immunoassays may not be able to differentiate an active from inactive (e.g. hydrolyzed or polymerized) antibiotic. In our study, the antibiotic concentrations appeared to fluctuate even though the samples were obtained from the same bag. Given acceptable quality assurance standards for the antibiotic assay [11], we used only one bag of each brand of PD solution per antibiotic for each storage condition. The reason for the apparent fluctuating concentrations may reflect sampling error (e.g. the solution in the injection port was inadequately mixed in some cases). Future studies may include multiple bags to control for this possibility.

The data regarding ampicillin stability and compatibility in the different PD solutions is interesting. According to the bioassay which was conducted at the time of sampling, very little ampicillin remained in the solution after 4 days. However, direct measurement of the concentration using HPLC at a later date failed to detect any ampicillin. This difference in results may relate to ongoing hydrolysis during defrosting and analysis of the sample. Alternatively, toxic hydrolytic products may have been formed transiently, and it was these products that caused some residual activity in the bioassay; this has not been previously reported for ampicillin but has been reported for other beta-lactam antibiotics.
Further investigation would be interesting to clarify the unexplained differences between the results of the bioassay and direct antibiotic measurement.

Factors influencing the stability and compatibility of ampicillin in intravenous and PD solutions have been researched. In intravenous solutions, the pH for maximal stability is approximately 5–6 and stability decreases in more alkaline solutions [13]. However, in general, these studies did not assess ampicillin stability for >48 h and they did not measure both bioactivity and antibiotic concentration. In our data, while the pH of the Gambro PD solution was higher than the Baxter PD solution, it was similar or less than the Fresenius solution in which ampicillin stability appeared reasonable. The change in pH in the Gambro PD solution following addition of antibiotics [9].

Fig. 1. Standard curves showing the relationship between bioactivity (median inhibition diameter and range) and antibiotic concentration.

Fig. 2. Influence of storage conditions and time on antibiotic bioactivity (median diameter and range of inhibition of bacterial growth).
ampicillin may suggest an *in vitro* chemical reaction that is contributing to the degradation of ampicillin. Alternatively, it is possible that ampicillin partitions at least in part into the plastic bag.

The PD solutions in this study were loaded with antibiotics in a sterile dispensing unit, so as expected, sterility of the solutions were maintained for the study duration. However, this method of loading PD solutions with antibiotics is not consistent across different Australian institutions. Anecdotal evidence suggests that in the majority of Australian PD units, solutions are loaded by PD nurses either in the patients’ homes or at hospital or, in some cases, patients are trained to load their own PD solutions with antibiotics. The results of this study have demonstrated antibiotic stability (with the exclusion of ampicillin in the Gambro solution) and sterility in the different PD solutions for up to 3 weeks, which is convenient for patients living in regional areas. However, the results of the sterility tests may only be applied to situations where the PD bags are loaded under sterile conditions.

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