Research Letter

High Pyridine Generation in Ceftazidime-Icodextrin Admixtures Used to Treat Peritoneal Dialysis-associated Peritonitis

Harpreet Kaur, BPharm[1]; Harmanjeet Harmanjeet, MPharmSc[1]; Troy Wanandy, MSc[1,2]; Ronald L. Castelino, PhD[3,4]; Kamal Sud, FRACP[4,5]; Matthew D. Jose, PhD[6]; Gregory M. Peterson, PhD[1]; and Rahul P. Patel, PhD[1]

[1]Department of Pharmacy, School of Medicine, University of Tasmania, Hobart, Tasmania, Australia; [2]Department of Pharmacy and Jack Jumper Allergy Program, Royal Hobart Hospital, Hobart, Tasmania, Australia; [3]Faculty of Medicine and Health, The University of Sydney, New South Wales, Australia; [4]Peritoneal Dialysis Unit, Regional Dialysis Centre, Blacktown Hospital, Blacktown, New South Wales, Australia; [5]Department of Renal Medicine, Nepean Hospital and Nepean Clinical School, The University of Sydney, Kingswood, New South Wales, Australia; and [6]Department of Nephrology, Royal Hobart Hospital, Hobart, Tasmania, Australia

ABSTRACT

Purpose: To investigate the amount of pyridine generated from degradation of ceftazidime in icodextrin peritoneal dialysis (PD) solutions.

Methods: PD solutions that contained 1 and 1.5 g of ceftazidime were stored at 25 °C for 12 hours and then at 37 °C for 14 hours. An aliquot was withdrawn at predefined time points and analyzed for the concentrations of ceftazidime and pyridine.

Findings: The amount of pyridine generated was >225% and 400% of its maximum recommended daily exposure in the 1- and 1.5-g ceftazidime-PD admixtures, respectively.

Implications: Until these results are confirmed with appropriate *in vivo* studies, intermittent intraperitoneal dosing of ceftazidime admixed with icodextrin should be used with caution and appropriate clinical monitoring or a suitable alternative antibiotic should be used. (*Clin Ther*. 2019;41:2446–2451)

Key words: ceftazidime, peritoneal dialysis, peritonitis, pyridine, stability, toxicity.

INTRODUCTION

Approximately 11% of the global dialysis population use peritoneal dialysis (PD) for the treatment of end-stage renal disease.[1] Icodextrin-containing solutions are used to promote ultrafiltration in patients receiving PD, while limiting exposure to high concentrations of dextrose, which has deleterious effects on long-term peritoneal membrane function.[2] The uptake of icodextrin use is increasing globally, with >50% patients receiving PD in the developed world using these solutions.[3] In patients receiving continuous ambulatory PD (CAPD), where exchanges are conducted manually, icodextrin is administered as a nighttime exchange with a long dwell time of up to 8–10 hours. Alternatively, in patients receiving automated PD (APD), where a cycler performs multiple PD exchanges at nighttime, icodextrin is administered during the long daytime dwell of up to 12–14 hours.[2,4] Despite the effectiveness of PD as a dialysis modality, PD-associated peritonitis (PDAP) is still a major cause of morbidity and mortality with this treatment,[5] and the need for icodextrin may also

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increase during an episode of peritonitis because of transient loss of ultrafiltration. Intermittent (once-daily) intraperitoneal administration of 20 mg/kg or 1−1.5 g of ceftazidime during a long dwell time is recommended for the treatment of PDAP caused by *Pseudomonas* species,[5,6] necessitating the consideration of using this drug in icodextrin-containing PD solutions.

Because modern APD machines can perform the function of infusing a long day-time dwell immediately after the last nighttime exchange, in nonhospitalized patients, ceftazidime-PD fluid admixtures are typically prepared >12 hours before their IP administration (ie, anytime during the day before beginning of the first APD cycle at night). This would also be true for patients receiving CAPD who are administered icodextrin for the nighttime exchange. Therefore, a prepared admixture can remain at room temperature for >12 hours and then at body temperature for up to 14 hours.

On degradation, ceftazidime produces pyridine, which has been reported to be neurotoxic and hepatotoxic in animals and humans.[7−11] The reported neurologic and hepatic effects of pyridine are largely based on animal studies because there is no valid rationale for intentional administration of pyridine in humans. Two patients with epilepsy developed hepatotoxicity when treated with pyridine.[9] However, the lack of information on their hepatic status before pyridine administration and the coadministration of other medications precluded a definite causative role of pyridine in the observed hepatic adverse effects. Nevertheless, hepatotoxic effects of pyridine in rats include liver enlargement, inflammatory hepatic lesions, and necrosis.[10] Pyridine is a central nervous system depressant, and its neurologic effects in patients with epilepsy are reported to include slow and slurred speech, slow reflexes, and a stuporous condition.[9] When exposed to undetermined amounts of pyridine vapors, healthy adults reported temporal headaches, sensations approaching giddiness, and a desire to sleep.[11]

Neurotoxicity in persons exposed to pyridine vapors at a concentration of 125 ppm for 4 hours per day for 1−2 weeks has been reported.[12] In a human study, 3.4 mg of ^{14}C-pyridine was orally administered in orange juice to 2 male participants.[13] The urinary recovery of unchanged pyridine and its metabolite, *N*-methylpyridinium (a compound more toxic than pyridine in animals[14]), was found to account for 67% and 8.5% of the administered pyridine dose, respectively, in a 24-hour period, indicating urinary excretion is the major route of elimination for these compounds. The recommended treatment for PDAP caused by *Pseudomonas aeruginosa* in patients with end-stage renal disease is intraperitoneal ceftazidime for 21 days,[5] resulting in the possibility of accumulation of these toxic compounds. Therefore, patients with PDAP treated with intraperitoneal ceftazidime-icodextrin admixture could be exposed to much higher than the maximum recommended limit of pyridine on a daily basis for the treatment period, possibly resulting in neurotoxicity.

Ceftazidime, when admixed with icodextrin PD solution, is reported to be stable at 25 °C (room temperature) and 37 °C (body temperature).[15,16] However, whether the amount of toxic pyridine generated because of degradation of ceftzadime in icodextrin solution exceeds the maximum recommended daily exposure (2 mg/d)[17] when kept at room temperature for 12 hours and then at body temperature for 14 hours remains to be determined. Therefore, the aim of this study was to investigate the amount of pyridine generated because of degradation of ceftazidime in icodextrin PD solution when stored at 25 °C for 12 hours and then at 37 °C for 14 hours.

METHODS

Sample Preparation

Ceftazidime powder for injection (n = 4, 2 g each, Juno Pharmaceuticals Pty Ltd, Melbourne, Victoria, Australia) was reconstituted with 10 mL of water for injection to obtain 200 mg/mL of ceftazidime. Then 5 or 7.5 mL of reconstituted solution was injected into 7.5% icodextrin PD bags (Baxter Pty Ltd, Sydney, New South Wales, Australia) to achieve 1 or 1.5 g per 2 L of ceftazidime. In total, 6 PD solutions with icodextrin (3 bags for each concentration of ceftazidime) were prepared and first stored at 25 °C for 12 hours and then at 37 °C for 14 hours. An aliquot was withdrawn at time 0 (baseline) and after 3, 6, 9, and 12 hours of storage at 25 °C and then after 2, 4, 6, 8, 10, 12, and 14 hours of storage at 37 °C. Each aliquot was then analyzed to determine the concentrations of ceftazidime and pyridine using a validated stability-indicating high-performance liquid chromatography (HPLC).

HPLC Analysis

HPLC was performed using a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Sunnyvale, California). The HPLC separation of ceftazidime pentahydrate and pyridine standards (Sigma−Aldrich, New South Wales, Australia) was performed using an ACE 5 μm C_{18} column (100 Å, 150 × 4.6 mm internal diameter, Advanced Chromatography Technologies, Aberdeen, Scotland). The gradient elution for mobile phase A (0.1% vol/vol trifluoracetic acid in water) to mobile phase B (0.1% vol/vol trifluoracetic acid in acetonitrile) was as follows: 98.5%:1.5% for the initial 3 minutes, 80%:20% for the next 3 minutes, 60%:40% for another 4 minutes, followed by 80%:20% for 2 minutes, and finally 98.5%:1.5% for the last 4 minutes. The flow rate and injection volume were set at 1 mL/min and 10 μL, respectively. The detector wavelengths were set at 254 and 270 nm for measurements of pyridine and ceftazidime, respectively.

The stability-indicating nature of the HPLC method was investigated by admixing ceftazidime solution (1 mg/mL in water) with an equal volume of 1 N hydrochloric acid (acidic stress) or 0.001 N sodium hydroxide (basic stress) for 1 hour. For thermal stress, the ceftazidime solution (500 μg/mL in water) was kept at 50 °C for 1 hour. Unstressed and stressed samples were analyzed for comparative purpose. The linearity of the method, estimated using the correlation coefficient r,[2] was investigated using 0, 50, 100, 200, 400, and 600 μg/mL of ceftazidime standard (Sigma−Aldrich) and 0, 0.2, 0.5, 1.25, 2.5, and 5 μg/mL of pyridine standard (Sigma−Aldrich). Mean intraday and interday precisions were investigated, with repeat analysis (n = 6) during 5 days, using peak area of 50, 200, and 600 μg/mL of ceftazidime and 0.5, 1.25, and 5 μg/mL of pyridine. Mean intraday and interday accuracy using the above-mentioned concentrations of ceftazidime and pyridine were calculated as follows: (observed concentration − expected concentration)/expected concentration × 100). Mean intraday and interday repeatability were determined using retention times of 1.25 and 200 μg/mL of ceftazidime and pyridine, respectively. An aliquot withdrawn from each PD bag was analyzed in duplicate to determine the concentration of ceftazidime and the generated amount of pyridine.

RESULTS

The chromatographic peak of unstressed ceftazidime eluted at 8.8 minutes. Acidic, basic, and thermal stress resulted in the loss of 9%, 20%, and 15% of ceftazidime, respectively. Depending on the type of stress condition, several degradation peaks were observed. Interestingly, a degradation peak representing pyridine was eluted at 2.5 minute with all types of stress conditions. Importantly, all the degradation products were completely separated and did not interfere with the peak of ceftazidime, suggesting the developed HPLC method is suitable for the stability analysis of ceftazidime. The standard curves were linear, with a correlation coefficient >0.99 for the range of 0−500 μg/mL and 0−5 μg/mL of ceftazidime and pyridine, respectively. Interday and intraday precision, accuracy, and repeatability were all <2.5%.

The mean (SD) concentrations of 1 and 1.5 g of ceftazidime in 2 L of icodextrin PD solution were 516 (2.5) and 835 (1.5) μg/mL, respectively, and were considered to be 100%. The percentage of ceftazidime remaining and the amount of pyridine generated before and after the storage of ceftazidime-icodextrin PD admixtures are shown in Figure 1. Ceftazidime (1 g) in PD solution retained 94.0% of its initial concentration when stored at 25 °C for 12 hours and then at 37 °C for another 14 hours (total of 26 hours of storage). The amount of pyridine generated in 1 g per 2 L of ceftazidime solution was >4.5 mg. The 1.5 g of ceftazidime in PD solution lost approximately 6.6% of its initial concentration when stored at 25 °C and then 37 °C for 12 and 14 hours, respectively. The amount of generated pyridine was 8.3 mg.

DISCUSSION

For intermittent dosing, antibiotic-containing PD solutions must be allowed to dwell for at least 6 hours for adequate absorption of the antibiotic.[5] Therefore, in patients receiving PD, intraperitoneal antibiotics need to be administered during the long dwell, which is usually during the daytime in APD and nighttime in CAPD. Because icodextrin PD solution is also recommended to be administered as a long dwell for it to be effective for ultrafiltration[2] and because the incidence of PDAP caused by *Pseudomonas aeruginosa* is increasing,[18,19] it not

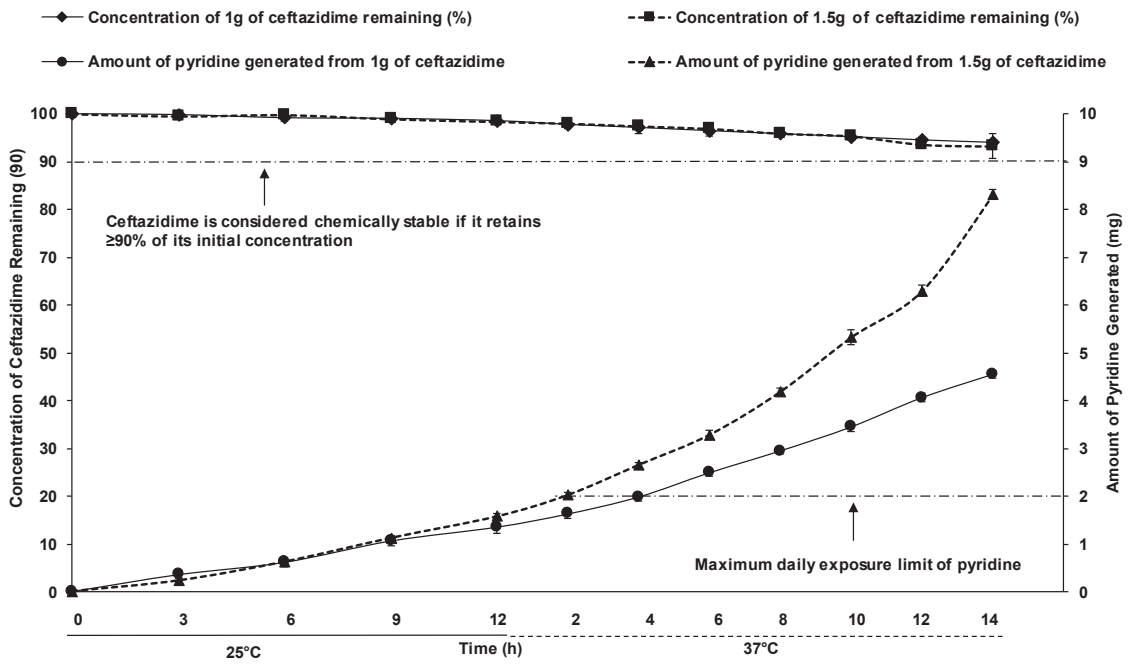

Figure 1. Percentage of ceftazidime remaining and amount of pyridine generated in ceftazidime-icodextrin peritoneal dialysis admixtures kept at 25 °C for 12 h and then at 37 °C for 14 h. Error bars represent SD.

uncommon to consider intraperitoneal administration of ceftazidime admixed with icodextrin PD solution. Ceftazidime-PD solution admixtures may be exposed to room and body temperatures for up to 12 and 14 hours, respectively, when used intermittently (every 24 hours) to treat *Pseudomonas* peritonitis.

This is the first *in vitro* study to investigate the amount of pyridine generated because of degradation of ceftazidime in icodextrin PD solutions. After the storage of 2-L PD admixtures for 26 hours (12 hours at 25 °C and then 14 hours at 37 °C), 1- and 1.5-g solutions of ceftazidime retained 94.0% and 93.4% of their initial concentrations, respectively, but the amount of generated pyridine was >225% (4.5 mg) and 400% (8.3 mg) of its maximum recommended daily exposure.

Cephalosporin-associated neurotoxic effects are often underestimated and overlooked.[20,21] Among various cephalosporins, ceftazidime is one of the most commonly reported antibiotics to cause central nervous system adverse reactions, including confusion, agitation, drowsiness and myoclonus, and the risk of such adverse effects is increased in patients with renal failure.[20,21] The mechanisms by which cephalosporins can cause neurotoxicity are not well understood, and their penetration through the blood brain barrier is poor. One proposed mechanism involves inhibition of the γ-aminobutyric acid (GABA) A receptor, resulting in decreased release of GABA and subsequent increase in excitatory neurotransmission.[22] However, a recent study suggested that the inhibition of GABA-A receptors is unlikely to be the primary cause of cephalosporin-associated neurotoxicity.[23] In addition, despite ceftazidime having a much lower affinity than cephazolin toward GABA-A, ceftazidime is more often reported to be neurotoxic than cephazolin.[20] Although glutaminergic excitation is another proposed mechanism for cephalosporin-induced neurotoxicity,[24] studies have found no affinity of ceftazidime to glutamate receptors.[22] The baseline pH of ceftazidime-icodextrin admixtures in the present study was slightly acidic (mean, 6.04). Under acidic conditions, the β-lactam ring of ceftazidime is opened

with simultaneous production of neurotoxic pyridine and methylene derivatives,[25] and therefore, the role of pyridine in ceftazidime-induced neurotoxicity cannot be ruled out.

To minimize exposure to pyridine in patients with PDAP treated with intermittent dosing of intraperitoneal ceftazidime, an alternative approach is to administer it via continuous dosing while patients are receiving CAPD. The infusion of low-dose ceftazidime in each PD bag potentially decreases the extent of pyridine formation because degradation of ceftazidime is also concentration dependent.[17] This approach cannot be used while patients are receiving APD because the continuous dosing dwell times with this modality are much shorter than the recommended 6 hours required for adequate absorption of the antibiotic. In addition, extrapolation of the continuous dosing regimen from CAPD to APD may result in underdosing because of increased peritoneal clearance and reduced absorption of intraperitoneal antibiotics associated with the rapid and frequent exchanges with short dwell times in APD.[5]

There are several limitations of extrapolating the *in vitro* results of this study into clinical practice. *In vivo* pyridine levels may be lower than what is reported here because *Pseudomonas* can biodegrade pyridine into ammonia,[12] which is also neurotoxic.[26] However, as the treatment progresses, the bacterial burden in the peritoneal cavity decreases; hence, biodegradation of pyridine is expected to decrease over time. In addition, because intraperitoneal ceftazidime is absorbed into the systemic circulation, less ceftazidime would be available during a prolonged period in the peritoneal cavity for subsequent degradation and pyridine generation. However, intraperitoneal ceftazidime has a biphasic distribution: from peritoneal fluid to plasma and then from plasma to peritoneal fluid.[27] Therefore, to what extent these *in vitro* results can be extrapolated *in vivo* is uncertain without conducting appropriate studies to determine the amount of pyridine present in the systemic circulation of patients. Until such studies are concluded, intraperitoneal intermittent dosing of ceftazidime admixed with icodextrin should be used with caution and appropriate clinical monitoring in patients with PDAP or a suitable alternative antibiotic should be used.

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Address correspondence to: Rahul P. Patel, PhD, Pharmacy, School of Medicine, University of Tasmania, Private Bag 26, Hobart, Tasmania 7001, Australia. E-mail: rahul.patel@utas.edu.au