Chemical and microbiological stability of diluted ceftazidime in three different solutions under three storage temperatures over a 28 day period

Sarah E. Hoff*, Darren J. Berger*, Austin K. Viall†, Dwayne Schrunk‡ and James O. Noxon*

*Department of Veterinary Clinical Sciences, Iowa State University, 1809 South Riverside Drive, Ames, IA 50011, USA
†Department of Veterinary Diagnostic Laboratory, Iowa State University, 1850 Christensen Dr, Ames, IA 50011, USA
‡Department of Veterinary Pathology, Iowa State University, 2784 Vet Med, Ames, IA 50011, USA

Correspondence: Sarah E. Hoff, Department of Veterinary Clinical Sciences, Iowa State University, 1809 South Riverside Drive, Ames, IA 50011, USA. E-mail: sehoff@iastate.edu

Background – Ceftazidime (CAZ) solutions are being used based on anecdotal reports for otitis externa complicated by multidrug-resistant *Pseudomonas aeruginosa* (MDR PA). The chemical and microbiological stability of these proposed compounded solutions have not been evaluated, and likely are affected by the diluent and storage duration or temperature.

Hypothesis/Objectives – Compounded CAZ solutions would show variable degradation dependent on diluent, time and temperature. The antimicrobial activity of the solutions would reflect changes in concentration and not alterations to the chemical compound.

Methods and materials – Ceftazidime was compounded with 100 mL 0.9% sodium chloride (NA+CAZ), 118 mL Triz-EDTA Aqueous flush (TE+CAZ) and 125 mL Douxo Micellar Solution (MI+CAZ). Aliquots of the solutions were stored at 25°C, 4°C and −20°C for 28 days. High-performance liquid chromatography was used to analyse CAZ recovery from compounded solutions at weekly intervals. A modified broth dilution technique was utilised to assess minimum inhibitory concentration (MIC) to monitor antimicrobial activity against a reference PA strain.

Results – Temperature, duration of storage and diluent each had independent effects on the chemical stability of CAZ. CAZ concentrations decreased over time as well as with increased temperature. NA+CAZ solutions exhibited the least degradation compared to the other solutions. The MIC for PA was most consistent for NA+CAZ solutions regardless of storage temperature and duration of storage.

Conclusions and clinical importance – Chemical and microbiological stability of compounded CAZ solutions varied by diluent, storage temperature and duration of storage. Dilution in NA resulted in the lowest variation in stability over 28 days when stored at refrigerated or frozen temperatures compared to other diluents.

Introduction

Chronic cases of canine otitis externa (OE) often develop infections with *Pseudomonas aeruginosa* (PA), which possesses a high intrinsic antibiotic resistance and can acquire new resistance very rapidly.\(^1,2\) Because of the resistant nature of chronic OE due to PA and the limited number of commercially available otic preparations available in the United States, many practitioners look to compounded medications for more antimicrobial choices. One antibiotic that has been recommended for topical use in cases of chronic OE due to PA is ceftazidime (CAZ).\(^3\)

Ceftazidime is a third-generation cephalosporin that has high activity against PA, even amongst canine strains of multidrug-resistant (MDR) PA.\(^4-6\) However, cephalosporins are known to have poor stability after reconstitution.\(^7\) Factors found to accelerate the degradation of CAZ in solution include exposure to UV light, increased temperature and pH outside the 4.5–6.5 range.\(^8-11\) Studies to date have evaluated the stability of CAZ over a matter of hours to days, primarily for use as an intravenous infusion. For cases of chronic OE, treatment often is prolonged, lasting several weeks, and the stability of CAZ in recommended topical solutions for this length of time is unknown.

The aim of our study was to determine and compare the chemical and microbiological stability of CAZ in three compounded solutions, stored under three temperature conditions, over the course of 28 days.\(^1,2,13\) We hypothesised that CAZ would be the most stable in diluents with an optimal pH for CAZ (4.5–6.5), that CAZ would degrade over time, and that CAZ would be more stable at lower temperatures. Our final hypothesis was that any change
in antimicrobial activity would be attributable to a reduced CAZ concentration.

**Methods and materials**

**Compounded ceftazidime solutions**

Conference proceedings and veterinary information-sharing networks were searched for clinical recommendations from ACVD-boarded veterinary surgeons advocating the use of compounded CAZ in cases of CE due to MDR PA. Based on the current clinical recommendations, the diluents and volumes used were as follows:

- 0.9% NaCl 100 mL (NA)(Baxter Healthcare Corporation; Deerfield, IL, USA); Tris-EDTA 118 mL (TE)(Triz-EDTA Aqueous flush, Dechra Veterinary Products; Leawood, KS, USA); Phytosphingosine HCL 0.02% 125 mL (MI)(Douxo Micellar Solution, Ceva Animal Health; Lenexa, KS, USA).

A commercially available 1 g CAZ powder for injection (Tazicef, Hospira Worldwide Inc.; Lake Forest, IL, USA) was used to prepare the solutions. The compounded solutions were formulated as follows:

1. NA+CAZ: 0.9% NaCl 100 mL + 1 g CAZ
2. TE+CAZ: Tris-EDTA 118 mL + 1 g CAZ
3. MI+CAZ: Phytosphingosine HCL 0.02% 125 mL + 1 g CAZ

CAZ was dissolved in each diluent using an aseptic technique, resulting in final concentrations of approximately 1% based on the volume of the diluent (1% for NA+CAZ, 0.85% for TE+CAZ and 0.8% for MI+CAZ). The solutions were gently rocked for 60 s to aid in dissolution. Two duplicate lots were made for each compounded solution, and each lot was divided into individual syringes of 1 mL aliquots and stored in opaque bags at 25°C, 4°C or -20°C within 30 min of preparation. Three samples were selected randomly from each lot and storage temperature for analysis at Day (D)0, D7, D14, D21 and D28. Control aliquots of each pure diluent (NA, TE, MI) also were stored at each temperature and analysed at each time point.

**pH determination**

The pHs of the compounded solutions, NA alone, TE alone and MI alone were determined using a Fisher Scientific Accumet AB15 Basic pH meter (ThermoFisher Scientific; Waltham, MA, USA) at all time points after calibration. Three aliquots of the compounded solutions were combined from an individual lot and storage temperature for pH measurement.

**Chemical stability**

The chemical stability of CAZ in the solutions was determined using high-performance liquid chromatography (HPLC) with a Dionex UltiMate 3000 HPLC System (ThermoFisher Scientific). The HPLC protocol was based on a previous study. Briefly, separation was carried out by reverse-phase chromatography on a Spherisorb C6, 80Å, 5 μm column (100 x 4.6 mm) with a mobile phase composed of 7% acetonitrile and 93% 0.05 μL ammonium acetate at a flow rate of 2 mL/min. The injection volume was 10 μL, and the detector wavelength was analysed at both 257 and 310 nm. The wavelengths were chosen based on previous data and lack of interference with the diluents.

A CAZ standard (ceftazidime pentahydrate, United States Pharmacopeia (USP) reference standard, Sigma-Aldrich; St Louis, MO, USA) was prepared by weighing 100 mg of the powder into a 5 mL volumetric flask and 1 mL of 1.2% aqueous sodium carbonate was added to aid in dissolution. The solution was brought to volume with sterile water, and a 100 μL aliquot was transferred to a 10 mL flask and brought to volume with sterile water, for a final concentration of 0.2 mg/mL. This was further diluted to generate a standard curve, which was repeated weekly before sample analysis. The lower limit of detection was 0.01 mg/mL based on sensitivity of the instrument. Samples were equilibrated to room temperature and a 100 μL aliquot of the compounded solution was diluted with sterile water to an approximate concentration of 0.1 mg/mL for HPLC analysis. Differences in peak area between the CAZ standard curve and sample curves were used to determine the percentage recovery.

**Antimicrobial activity and minimum inhibitory concentration determination**

The bacterial strain used in this study was Pseudomonas aeruginosa (ATCC 27853) from American Type Culture Collection (ATCC). After 24 h incubation at 37°C, colonies on blood agar plates were added to 0.9% phosphate buffered saline and suspensions were adjusted to 0.5 McFarland standard turbidity. The standardised 0.5 McFarland bacterial suspension was diluted 1:1000 in Mueller-Hinton broth (MH, Becton Dickinson & Co.; Sparks, MD, USA) by transferring a CAZ standard [ceftazidime pentahydrate, United States Pharmacopeia (USP) reference standard, Sigma-Aldrich; St Louis, MO, USA] to aid in dissolution. The solution was brought to volume with sterile water, and a 100 μL aliquot was transferred to a 10 mL flask and brought to volume with sterile water, for a final concentration of 0.2 mg/mL. This was further diluted to generate a standard curve, which was repeated weekly before sample analysis. The lower limit of detection was 0.01 mg/mL based on sensitivity of the instrument. Samples were equilibrated to room temperature and a 100 μL aliquot of the compounded solution was diluted with sterile water to an approximate concentration of 0.1 mg/mL for HPLC analysis. Differences in peak area between the CAZ standard curve and sample curves were used to determine the percentage recovery.

**Figure 1.** Relative ceftazidime concentrations (RCCs) at three different storage temperatures over time.

RCC (a) at room temperature (25°C), (b) at refrigerated temperature (4°C) and (c) at frozen temperature (-20°C) with respect to time. Data presented as mean RCC ± standard deviation. Triz, 118 mL Triz-EDTA Aqueous flush + 1 g Ceftazidime (CAZ) compounded solution; Micellar, 125 mL Douxo Micellar Solution + 1 g CAZ compounded solution; NaCl, 100 mL 0.9% NaCl + 1 g CAZ compounded solution. *RCC was below limit of detection (0.01 mg/mL) of the high-performance liquid chromatography assay.
Diluted ceftazidime stability

Table 1. Mean relative ceftazidime concentrations (RCCs) at 25°C, 4°C and −20°C over time, compared to Day (D)0. Data presented as mean ± standard deviation.

| Solution | Storage temperature | D7 | D14 | D21 | D28 |
|----------|---------------------|----|-----|-----|-----|
| TE+CAZ   | 25°C                | 0.27 ± 0.01 | 0.13 ± 0.01 | * | * |
|          | 4°C                 | 0.67 ± 0.01 | 0.48 ± 0.02 | 0.35 ± 0.01 | 0.25 ± 0.01 |
|          | −20°C               | 0.25 ± 0.02 | 0.11 ± 0.04 | 0.11 ± 0.01 | 0.08 ± 0.01 |
| MI+CAZ   | 25°C                | 0.53 ± 0.02 | 0.42 ± 0.02 | 0.34 ± 0.03 | * |
|          | 4°C                 | 0.89 ± 0.07 | 0.72 ± 0.05 | 0.75 ± 0.06 | 0.65 ± 0.02 |
|          | −20°C               | 0.90 ± 0.05 | 0.84 ± 0.02 | 0.84 ± 0.03 | 0.91 ± 0.04 |
| NA+CAZ   | 25°C                | 0.83 ± 0.02 | 0.60 ± 0.04 | 0.53 ± 0.03 | 0.40 ± 0.01 |
|          | 4°C                 | 0.98 ± 0.05 | 0.91 ± 0.07 | 0.89 ± 0.01 | 0.81 ± 0.02 |
|          | −20°C               | 0.83 ± 0.07 | 0.76 ± 0.13 | 0.84 ± 0.04 | 0.85 ± 0.08 |

*Below limit of detection (0.01 mg/mL) of the high-performance liquid chromatography assay.

10 µL of the 0.5 McFarland bacterial suspension to 10 mL of MHB. This resulted in a final concentration of approximately 1.5 × 10^8 colony forming units/mL.

The minimum inhibitory concentration (MIC) of the compounded solutions was determined using a modified broth microdilution method published previously.16,17 Briefly, each well of a 96 well microtiter plate was filled with 50 µL MHB. The first well of each plate was a negative control and no bacterial suspension was added. The second well was a positive control and no CAZ solution was added. The third well represented the first sampling well, and the MHB was removed and discarded. In its place, 100 µL of the compounded CAZ solution was added. Serial two-fold dilutions were performed by transferring 50 µL from the first well to the second and each subsequent well to a final dilution of 1:8192. From the final well, 50 µL was removed and discarded. Three replicated samples were made for each lot, storage temperature and diluent. Dilutions of the pure diluent were carried out in the same manner to a final dilution of 1:16. Following the dilutions, 50 µL of the bacterial suspension was added to each well except the negative control.

Anticipated starting concentrations of the compounded CAZ solutions ranged from 0.5 mg/mL to 7.6 × 10^5 mg/mL. Starting concentrations were calculated based on the HPLC concentration for the sample and two-fold dilutions. The microtiter plates were incubated for 24 h at 37°C and each well was inspected for visible growth of PA. MIC was defined as the last dilution that resulted in no visible growth of bacteria.

Statistical analysis

Normality of the data was assessed via a D’Agostino–Pearson analysis. The coefficient of variation (CV) for HPLC-based measurements was calculated from replicates for each time point and temperature for each concentration; CV <10% was deemed acceptable analytical precision. A two-way, repeated measures ANOVA was performed, evaluating the effects of time and storage temperature on the pH of solutions and CAZ concentration; separate analyses were performed for each compounded solution. Differences in pH between the three solutions were performed for each time point using a one-way ANOVA with Tukey’s correction for multiple comparisons ANOVA. For evaluating differences in concentrations between the formulations, concentrations at each time point for each storage temperature were converted to relative ceftazidime concentration (RCC) using the following equation:

\[
RCC = \frac{\text{Recovered ceftazidime (mg)}}{\text{Initial ceftazidime concentration (mg)}}
\]

Conversion to RCC for subsequent analyses was needed as the starting concentrations of the CAZ were different between the three solutions. At each temperature and time point, the RCC of solutions was compared using a one-way ANOVA with Tukey’s correction for multiple comparisons. A Spearman correlation analysis was performed to evaluate correlation between pH and concentration for each solution. For assessing the effects of time and storage temperature on MIC, a two-way repeated measures ANOVA was performed; separate analyses were performed for each solution. Statistical significance was set at \( P < 0.05 \). Statistical analyses were performed using commercially available software (PRISM 8, Graphpad Software LLC; San Diego, CA, USA).

Results

pH determination

The initial pHs were 5.29 for NA, 6.29 for MI and 8.26 for TE, and 6.49 for NA+CAZ, 6.11 for MI+CAZ and 8.13 for TE+CAZ. For NA+CAZ and MI+CAZ solutions, the maximal pH change from original pH to end of study pH was <0.2; for TE+CAZ the maximal change was <0.65. At all time points, the pH for MI+CAZ was lower than the pH for NA+CAZ and TE+CAZ (\( P < 0.0001 \)); there were no differences in pH between NA+CAZ and TE+CAZ. For NA+CAZ, there was no correlation between pH and drug concentration (\( r = 0.24, P = 0.03 \)) yet there was a moderate correlation between pH and concentration for MI+CAZ (\( r = 0.66, P < 0.001 \)), and strong correlation between pH and concentration for TE+CAZ (\( r = 0.90, P < 0.001 \)).

Chemical stability

The CV of CAZ measurements ranged from 2.3% to 8.7%. Storage temperature, storage duration and diluent each had independent significant effects on the RCC (see Figure 1 and Table 1). All solutions, regardless of storage temperature, showed increased degradation over time. Room temperature samples showed increased degradation of CAZ compared to refrigerated and frozen samples for all diluents. There was no significant difference in RCC between frozen samples for NA+CAZ and MI+CAZ at any time point (\( P > 0.40 \)). By D7, there was a significantly lower RCC for TE+CAZ samples stored frozen compared to those stored at refrigerated temperatures (\( P < 0.001 \)).

At any given time point and storage temperature, the RCCs for TE+CAZ solutions were significantly lower than those for NA+CAZ and MI+CAZ solutions. Likewise, the RCCs for MI+CAZ solutions were significantly lower than those for NA+CAZ for room temperature and refrigerated samples, and no significant difference was noted between frozen samples.
Antimicrobial activity and MIC
PA growth was inhibited by TE alone and MI alone at the first dilution, and not inhibited by NA alone at any dilution. We were unable to calculate the MIC of the compounded CAZ solutions for room temperature samples at D21 for TE+CAZ, and D28 for both TE+CAZ and MI+CAZ owing to loss of detectable CAZ on the HPLC assay (see Figure 2 and Table 2).

At D0, the MIC of NA+CAZ for PA was not significantly different from those for TE+CAZ or MI+CAZ, while TE+CAZ solutions had a significantly lower MIC compared to MI+CAZ solutions (P = 0.007). There was no significant difference between the MIC of the NA+CAZ and TE+CAZ samples stored at the same temperature at any time point except D28 under frozen conditions, when the MIC of NA+CAZ was significantly higher than that of TE+CAZ for PA.

Neither temperature nor time had significant effects on the MIC of the NA+CAZ solutions for PA. Both temperature and time had effects on the MIC of MI+CAZ for PA. For room temperature samples, the MIC of the MI+CAZ solutions significantly increased over time from D0 to D21 (5.07 ± 0 µg/mL versus 6.29 ± 1.40 µg/mL, P < 0.001). The MIC of the MI+CAZ solutions was significantly lower at D7, D21 and D28 for refrigerated solutions, and at D7 and D14 for frozen samples compared to D0. The MIC of the MI+CAZ solutions stored at room temperature was significantly higher than refrigerated or frozen samples at any time point.

Only time had a significant effect on the MIC of TE+CAZ for PA. The TE+CAZ samples stored at room temperature had a significant increase in MIC from D0 to D14 (2.37 ± 0 µg/mL versus 5.79 ± 2.03 µg/mL, P = 0.04). For samples stored at frozen temperatures, the MIC significantly increased from D0 to D28 (2.37 ± 0 µg/mL versus 3.17 ± 0 µg/mL, P < 0.001). There was no significant difference between the MIC at any given time point between storage temperature for available TE+CAZ solutions.

Discussion
This study shows that compounded CAZ solutions have variability in the chemical stability of CAZ, influenced by storage duration, storage temperature and base diluent. Furthermore, this study shows that the microbiological stability of CAZ also may be altered based on these variables. The diluent that had the least amount of variability in its chemical and microbiological stability was NA.

Previous studies have demonstrated that changes in pH influence CAZ stability.\(^{8,11}\) In this study, pH was only significantly correlated with RCC for MI+CAZ and TE+CAZ solutions; this suggests that factors other than pH also influenced the stability of CAZ in these solutions. The diluent itself was associated with changes in RCC as CAZ was the least stable in TE+CAZ solutions. Across all diluents and time points, CAZ was the least stable at room temperature. Overall, decreasing temperature resulted in increased stability with the exception of TE+CAZ. At this time, it is unknown why compounded TE+CAZ appears to be less stable when frozen. However, with regard to saline, there was no statistically significant difference in the final RCC at D28 between refrigerated and frozen samples. The MI+CAZ solution exhibited significant degradation of CAZ at 25°C and 4°C by D28. However, the RCC of MI+CAZ stored at –20°C was not statistically different from that of NA+CAZ at –20°C for any time point.

The MIC of the CAZ solutions for PA differed between diluent and was affected by both storage duration and conditions. However, for NA+CAZ solutions, neither storage temperature nor duration affected MIC. This suggests that the interaction between the variables can alter the microbiological activity of CAZ when compounded.

Figure 2. Minimum inhibitory concentration (MIC; µg/mL) of compounded ceftazidime (CAZ) solutions for Pseudomonas aeruginosa (ATCC 27853) over time at three different storage temperatures. (a) 100 mL 0.9% NaCl + 1 g CAZ (NA+CAZ), (b) 118 mL Triz-EDTA Aqueous flush + 1 g CAZ (TE+CAZ), (c) 125 mL Douxo Micellar Solution + 1 g CAZ (MI+CAZ). Data presented as mean MIC ± standard deviation. RT (room temperature), 25°C; Refrigerated, 4°C; Frozen, –20°C. *MIC was unable to be determined at Day 21 for TE+CAZ, and Day 28 for TE+CAZ and MI+CAZ owing to a loss of detectable CAZ on the high-performance liquid chromatography assay.
with MI or TE. The specific mechanism for this alteration with MI and TE, and the reason for the variability over time in these diluents is unknown.

One of CAZ’s primary mechanisms of action is via inhibition of penicillin-binding proteins, particularly penicillin-binding protein-3, which is located in the cytoplasmic domain of the bacterial envelope.\(^1\) It is known that TE increases the susceptibility of Gram-negative bacteria to certain antimicrobials through destabilisation of the bacterial cell wall.\(^2\) This action allows antibiotics such as CAZ to reach the cytoplasmic membrane more easily and exert its effects on the penicillin-binding proteins. Furthermore, TE has some intrinsic antibacterial properties.\(^3\) This phenomenon would explain the lower initial MIC of compounded CAZ solutions for PA. Phytosphingosine, the principal active ingredient in MI, has not been shown to have any antimicrobial activity as documented by the modified MIC assay. This demonstrated that the compounded solutions with measurable CAZ maintained microbiological stability. As this was an in vitro study, the therapeutic and toxicological stabilities were not evaluated.

This study had several limitations. One was that only three diluents were tested. It is not known how stable CAZ would be in other diluents or how the addition of other ingredients would affect stability. Due to the presence of inflammatory changes in OE, many practitioners also may consider the addition of a steroid such as dexamethasone to the compounded solution. As dexamethasone sodium phosphate has a pH of 7.0–8.5, which is above the optimal range for CAZ, our hypothesis is that its addition would potentially further decrease stability. Future studies could analyse the stability when additional products are added or in other diluents. A second limitation is that it is not known how the compounded CAZ solutions would perform in vivo. While the majority of compounded solutions maintained antimicrobial activity to D28, the susceptibility was carried out against a strain of PA that was highly susceptible to CAZ. This may not reflect the microbiological activity of compounded CAZ solutions in vivo, as other strains of PA are much more resistant.

In summary, the chemical and microbiological stability of compounded CAZ solutions is influenced by diluent, storage temperature and duration of storage. This variability was the lowest when CAZ was diluted in NA and stored at refrigerated or frozen temperatures. Therefore, when compounded CAZ is considered for topical use in cases of MDR PA, it should be diluted in NA and stored at refrigerated or frozen temperatures.

Acknowledgments

The authors thank Karen L. Campbell for providing a critical review of this manuscript.

Table 2. Average minimum inhibitory concentration (MIC, µg/mL) of compounded ceftazidime otic solutions for Pseudomonas aeruginosa (ATCC 27853) over time at three different storage temperatures (25°C, 4°C, –20°C). Data presented as mean MIC (µg/mL) ± standard deviation.

| Solution | Storage temperature | D0            | D7            | D14           | D21           | D28           |
|----------|---------------------|---------------|---------------|---------------|---------------|---------------|
| TE+CAZ   | 25°C                | 2.37 ± 0      | 3.81 ± 1.39   | 5.79 ± 2.03   | *             | *             |
|          | 4°C                 | 2.37 ± 0      | 3.19 ± 0      | 4.59 ± 3.56   | 3.05 ± 1.10   | 3.6 ± 1.31    |
|          | –20°C               | 2.37 ± 0      | 4.03 ± 1.25   | 2.88 ± 1.12   | 3.24 ± 1.18   | 3.17 ± 0      |
| MI+CAZ   | 25°C                | 5.07 ± 0      | 5.33 ± 0      | 7.08 ± 5.14   | 6.29 ± 1.40   | *             |
|          | 4°C                 | 5.07 ± 0      | 3.94 ± 0      | 4.24 ± 1.48   | 3.79 ± 0      | 3.3 ± 0       |
|          | –20°C               | 5.07 ± 0      | 4.56 ± 0      | 4.25 ± 0      | 3.89 ± 0.86   | 5.37 ± 1.88   |
| NA+CAZ   | 25°C                | 3.04 ± 1.67   | 2.94 ± 1.03   | 3.63 ± 0      | 3.19 ± 0      | 4.83 ± 0      |
|          | 4°C                 | 3.04 ± 1.67   | 3.48 ± 1.22   | 3.22 ± 1.13   | 3.15 ± 0.68   | 4.35 ± 1.11   |
|          | –20°C               | 3.04 ± 1.67   | 2.95 ± 1.03   | 3.08 ± 1.03   | 3.43 ± 1.33   | 4.73 ± 1.05   |

TE+CAZ, 118 mL Triz-EDTA Aqueous flush + 1 g Ceftazidime compounded solution (CAZ); MI+CAZ, 125 mL Douxo Micellar Solution + 1 g CAZ; NA+CAZ, 100 mL 0.9% NaCl + 1 g CAZ.

*Unable to be calculated owing to loss of detectable CAZ on the high-performance liquid chromatography assay.
References

1. Li XZ, Livermore DM, Nikaido H. Role of efflux pumps in intrinsic resistance of Pseudomonas aeruginosa: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob Agents Chemother 1994; 38: 1732–1741.

2. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med 2002; 95(Suppl 41): 22–26.

3. Fadok V. Otitis Externa: The Bane of Our Existence. Western Veterinary Conference 2013. Available at: https://studyres.com/doc/7874676/otitis-externa-the-bane-of-our-existence Accessed May 18, 2021.

4. Mekić S, Matanović K, Šeol B. Antimicrobial susceptibility of Pseudomonas aeruginosa isolates from dogs with otitis externa. Vet Rec 2011; 169: 125.

5. Seol B, Naglić T, Madić J, et al. In vitro antimicrobial susceptibility of 183 Pseudomonas aeruginosa strains isolated from dogs to selected antisepsidemonal agents. J Vet Med B Infect Dis Vet Public Health 2002; 49: 188–192.

6. van Klingerer B. An in vitro comparison of new cephalosporins with special reference to Pseudomonas aeruginosa. J Antimicrob Chemother 1981; B6(Suppl B): 97–106.

7. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. Clin Infect Dis 1998; 27: S93–99.

8. Jaruratanasirikul S, Sriwiriyajan S. Stability of ceftazidime in norfloxacin and storage temperature on physicochemical and microbiological formulation. J Clin Pharm Ther 2012; 28, e6.

9. Hayes MV, Orr DC. Mode of action of ceftazidime: affinity for the penicillin-binding proteins of Escherichia coli K12, Pseudomonas aeruginosa and Staphylococcus aureus. J Antimicrob Chemother 1983; 12: 119–126.

10. Yang Z, Qi HH, Zhang YL, et al. In vitro antibacterial activity of seven adjuvants against common pathogens associated with canine otitis externa. Vet Dermatol 2019; 30: 133-e138.

11. Li XZ, Livermore DM, Nikaido H. Role of efflux pump(s) in intrinsic resistance of Pseudomonas aeruginosa: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob Agents Chemother 1994; 38: 1732–1741.

12. Boyd M, Santoro D, Gram D. In vitro antimicrobial activity of topical otological antimicrobials and Tris-EDTA against resistant Staphylococcus pseudintermedius and Pseudomonas aeruginosa isolates from dogs. Vet Dermatol 2019; 30: 139-e140.

13. Buckley LM, McEwan NA, Nuttall T. Tris-EDTA significantly enhances antibiotic efficacy against multidrug-resistant Pseudomonas aeruginosa in vitro. Vet Dermatol 2013; 24: 519-e122.

14. Sparks TA, Kemp DT, Wooley RE, et al. Antimicrobial effect of combinations of EDTA-Tris and amikacin or neomycin on the microbiological resistance mechanisms of canine ears. Vet Dermatol 2019; 30: 218-e267.

15. Pye CC, Yu AA, Weese JS. Evaluation of biofilm production by Pseudomonas aeruginosa from canine ears and the impact of biofilm on antimicrobial susceptibility in vitro. Vet Dermatol 2013; 24: 446-e499.

16. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals – 4th ed. VET01S. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

17. Larsrupom L, Rungroj N, Leckharoensuk C, et al. In vitro antibacterial activity of mangosteen (Garcinia mangostana Linn.) crude extract against Staphylococcus aureus and Pseudomonas aeruginosa isolates from canine pyoderma. Vet Dermatol 2019; 30: 487-e145.

18. Tanner CL, Drake DR, Dawson DV, et al. Antibacterial activity of sphingolipid bases and fatty acids against Gram-positive and Gram-negative bacteria. Antimicrob Agents Chemother 2012; 56: 1157–1161.

19. Boyd M, Santoro D, Gram D. In vitro antimicrobial activity of topical otological antimicrobials and Tris-EDTA against resistant Staphylococcus pseudintermedius and Pseudomonas aeruginosa isolates from dogs. Vet Dermatol 2019; 30: 139-e140.

20. Buckley LM, McEwan NA, Nuttall T. Tris-EDTA significantly enhances antibiotic efficacy against multidrug-resistant Pseudomonas aeruginosa in vitro. Vet Dermatol 2013; 24: 519-e122.

21. Sparks TA, Kemp DT, Wooley RE, et al. Antimicrobial effect of combinations of EDTA-Tris and amikacin or neomycin on the microbiological resistance mechanisms of canine ears. Vet Dermatol 2019; 30: 218-e267.

22. Boyd M, Santoro D, Gram D. In vitro antimicrobial activity of topical otological antimicrobials and Tris-EDTA against resistant Staphylococcus pseudintermedius and Pseudomonas aeruginosa isolates from dogs. Vet Dermatol 2019; 30: 139-e140.

23. Buckley LM, McEwan NA, Nuttall T. Tris-EDTA significantly enhances antibiotic efficacy against multidrug-resistant Pseudomonas aeruginosa in vitro. Vet Dermatol 2013; 24: 519-e122.

24. Sparks TA, Kemp DT, Wooley RE, et al. Antimicrobial effect of combinations of EDTA-Tris and amikacin or neomycin on the microbiological resistance mechanisms of canine ears. Vet Dermatol 2019; 30: 218-e267.

25. Fennegan S, Percival SL. EDTA: an antimicrobial and antibiofilm agent for use in wound care. Adv Wound Care 2015; 4: 415–421.

26. Robinson VH, Paterson S, Bennett C, et al. Biofilm production of Pseudomonas spp. isolates from canine otitis in three different enrichment broths. Vet Dermatol 2019; 30: 218-e267.

27. Pye CC, Yu AA, Weese JS. Evaluation of biofilm production by Pseudomonas aeruginosafort canine ears and the impact of biofilm on antimicrobial susceptibility in vitro. Vet Dermatol 2013; 24: 446-e499.

28. United States Pharmacopeia. Stability considerations in dispensing practice. In: USP National Formulary (USP 39-NF 34). Rockville, MD, USA: United States Pharmacopeia, 2015; 1,191–1,198.

RÉSUMÉ

Contexte – Les solutions de ceftazidime (CAZ) ont été utilisées à partir de descriptions anecdotiques pour le traitement d’otites externes complicées par Pseudomonas aeruginosa multi-résistante (MDR PA). La stabilité chimique et microbiologique de ces solutions composées n’a pas été évaluée et pourrait être affectée par la dilution, et la durée et la température de stockage.

Hypothèses/Objectifs – Les solutions composées de ceftazidime (CAZ) pourraient présenter une dégradation variable en fonction du diluant, de la durée et de la température. L’activité antimicrobienne des solutions pourrait refléter les changements de concentration et pas l’altération de composition chimique.

Méthodes – La ceftazidime a été mélangée avec 100 mL de 0.9% sodium chloride (NA+CAZ), 118 mL Triz-EDTA Aqueous flush (TE+CAZ) et 125 mL Douxo Micellar Solution (MI+CAZ). Des aliquots de solutions ont été stockés à 25°C, 4°C et –20°C pour 28 jours. Une chromatographie liquide à haute performance a été utilisée pour analyser la récupération de CAZ à partir de solutions de mélanges toutes les semaines. Une technique de dilution a été utilisée pour déterminer la concentration minimale inhibitrice (MIC) pour montrer l’activité antimicrobienne contre la souche PA de référence.
Résultats – La température, la durée du stockage et le diluant de chacun avaient des effets indépendants sur la stabilité du CAZ. Les concentrations de CAZ ont diminuées au cours du temps ainsi qu’avec l’augmentation de température. Les solutions de NA+CAZ ont montré la dégradation la moins importante comparé aux autres solutions. La MIC pour PA n’était pas plus compatible avec les solutions de NA+CAZ sans lien avec la température et la durée du stockage.

Conclusions et importance clinique – La stabilité microbiologique et chimique des solutions de CAZ composées varie avec le diluant, la température et la durée de stockage. La dilution au NA a résulté en une variation plus faible sur 28 jours quand stockée à des températures réfrigérées ou congelées comparé aux autres diluants.

Resumen
Introducción – las soluciones de ceftazidima (CAZ) se están utilizando en base a informes anecdóticos para la otitis externa complicada con Pseudomonas aeruginosa multirresistente (MDR PA). No se ha evaluado la estabilidad química ni microbiológica de las soluciones compuestas propuestas, y es probable que se vean afectadas por el diluyente y la duración o temperatura de almacenamiento.

Hipótesis.Objetivos – las soluciones CAZ compuestas mostrarían una degradación variable en función del diluyente, el tiempo y la temperatura. La actividad antimicrobiana de las soluciones reflejaría cambios en la concentración y no alteraciones del compuesto químico.

Métodos – la ceftazidima se combinó con 100 ml de cloruro de sodio al 0,9% (NA + CAZ), 118 ml de Triz-EDTA enjuague acuoso (TE + CAZ) y 125 ml de solución micelar Douxo (MI + CAZ). Se almacenaron al cuarto de las soluciones a 25°C, 4°C y -20°C durante 28 días. Se utilizó cromatografía líquida de alta resolución para analizar la recuperación de CAZ de las soluciones compuestas a intervalos semanales. Se utilizó una técnica de dilución de caldo modificado para evaluar la concentración inhibitory mínima (MIC) para controlar la actividad antimicrobiana frente a una cepa de PA de referencia.

Resultados – la temperatura, la duración del almacenamiento y el diluyente tuvieron efectos independientes sobre la estabilidad química de CAZ. Las concentraciones de CAZ disminuyeron con el tiempo y también con el aumento de temperatura. Las soluciones NA + CAZ exhibieron la menor degradación en comparación con otras soluciones. El MIC para PA fue más consistente para soluciones NA + CAZ independientemente de la temperatura de almacenamiento y la duración del almacenamiento.

Conclusiones e importancia clínica – la estabilidad química y microbiológica de las soluciones CAZ compuestas varía según el diluyente, la temperatura de almacenamiento y la duración del almacenamiento. La dilución en NA resultó en la variación más baja en la estabilidad durante 28 días cuando se almacenó a temperaturas refrigeradas o congeladas en comparación con otros diluyentes.

Zusammenfassung
Hintergrund – Ceftazidime (CAZ) Lösungen werden nach anekdotischen Berichten für Otitis externa, welche durch Multi-Drug resistente Pseudomonas aeruginosa (MDR PA) verkompliziert werden, eingesetzt. Die chemische und mikrobiologische Stabilität dieser vorgeschlagenen Compounded (zusammengesetzten) Lösungen ist noch nicht evaluiert worden und wird vermutlich durch die Verdünnung und die Lagerdauer oder die Temperatur beeinflusst.

Hypothese/Ziele – Compounded CAZ Lösungen könnten abhängig von Verdünnungsmittel, Zeit und Temperatur einen unterschiedlichen Zerfall zeigen. Die antimikrobielle Aktivität der Lösungen könnte Veränderungen der Konzentration reflektieren und nicht Veränderungen der chemischen Zusammensetzung.

Methoden – Ceftazidime wurde aus 100 ml 0,9%iger Kochsalzlösung (NA+CAZ), 118 ml Triz-EDTA wässriger Spüllösung (TE+CAZ) und 125 ml Douxo micellärer Lösung (MI+CAZ) zusammengesetzt. Aliquote Mengen dieser Lösungen wurden bei 25°C, 4°C und -20°C 28 Tage lang gelagert. Die Hochleistungsflüssigkeitschromatografie wurde verwendet, um die Beständigkeit von CAZ in den zusammengesetzten Lösungen in wöchentlichen Intervallen festzustellen. Es wurde eine modifizierte Brühe-Mikroverdünnung verwendet, um die minimale Hemmstoffkonzentration (MIC) zur Überwachung der antimikrobiellen Aktivität gegenüber einem PE Referenzstamm zu erfassen.

Ergebnisse – Die Temperatur, Lagerungsdauer und Verdünnungsmittel zeigten voneinander unabhängige Einflüsse auf die chemische Stabilität von CAZ. Die CAZ Konzentrationen nahmen mit der Zeit sowie mit zunehmender Temperatur ab. NA+CAZ Lösungen zeigten die geringste Degradiation im Vergleich zu anderen Lösungen. Die MIC für PA war am beständigsten bei NA+CAZ Lösungen, wobei die Lagerungsstemperatur und die Lagerungsdauer dabei keine Rolle spielten.

Schlussfolgerungen und klinische Bedeutung – Die chemische und mikrobielle Stabilität von zusammengesetzten CAZ Lösungen unterschied sich je nach Verdünnungsmittel, Lagerungstemperatur und Lagerungsdauer. Im Vergleich zu anderen Verdünnungen varierte mit einer NA Verdünnung die Stabilität innerhalb von 28 Tagen am wenigsten, wenn sie bei gekühlten oder gefrorenen Temperaturen gelagert wurde.
摘要
背景 — 多剂耐性綠膿菌 (MDR PA) により復雑化した外耳炎に対して, Ceftazidime (CAZ) 溶液が使用されてるという逸話をある. これらの提案されている薬剤の化学的および微生物学的安定性は評価されておらず, おそらく溶液や保存期間や温度に影響されると思われる。
仮説/目的 — CAZの複数溶液は, 溶媒, 時間, 温度に依存して様々な劣化を示すであろう。溶液の抗菌活性は, 化合物の変化ではなく濃度の変化を反映していると考えられる。
方法 — Ceftazidime は, 100 mL の 0.9%塩化ナトリウム (NA+CAZ), 118 mL の Triz-EDTA 水溶液 (TE+CAZ) および 125 mL の Douxo Micellar Solution (MI+CAZ) と混合した。これらの溶液のアクリオート, 25°C, 4°C, -20°C で28日間保存した。速溶体クロマトグラフィー法を用いて, 1週間ごとに混合溶液からのCAZの回収率を分析した。修正した液体希釈法を用いて最小発育阻止濃度 (MIC) を評価し, 基準PM株に対する抗菌活性をモニターした。
結果 — AZの化学的安定性には, 溶度, 保存期間, 溶媒がそれぞれ独立した影響を及ぼした。CAZ濃度は, 温度の上昇, 時間の経過により低下した。NA+CAZ溶液は, 他の溶液に比べて最も劣化が少なかった。PAのMICは, 保存温度や保存期間にかかわらず, NA+CAZ溶液が最も安定していた。
結論および臨床上の重要性 — CAZ溶液の化学的および微生物学的安定性は, 溶媒, 保存温度および保存期間によって異なる。NAで希釈した場合, 冷蔵または冷凍で保存した場合の28日間の安定性の変動は, 他の希釈剤と比較して最も小さかった。

Resumo
Contexto — As soluções de ceftazidime (CAZ) estão sendo utilizadas na terapêutica de casos de otite externa complicada por Pseudomonas aeruginosa multirresistente (MDR PA) com base em relatos anedóticos. A estabilidade química e microbiológica dessas soluções manipuladas ainda não foi avaliada e provavelmente será afetada pelo diluente e pelo tempo de armazenamento ou temperatura.
Hipótese/Objetivos — Soluções de CAZ manipuladas sofreriam degradação variável dependente do diluente, tempo e temperatura. A atividade antimicrobiana das soluções seria influenciada por mudanças na concentração e não alterações no composto químico.
Métodos — A ceftazidima foi manipulada com 100 mL de cloreto de sódio (NA+CAZ), 118 mL de Triz-EDTA Flush aquoso (TE+CAZ) e 125 mL de Solução Micelar Douxo (MI+CAZ). Aliquotas das soluções foram armazenadas a 25°C, 4°C e -20°C por 28 dias. A cromatografia líquida de alto desempenho foi usada para analisar a recuperação de CAZ de soluções manipuladas em intervalos semi-anais. Uma técnica de diluição em caldo modificada foi utilizada para avaliar a concentração inibitória mínima (MIC) para monitorar a atividade antimicrobiana contra uma cepa de PA de referência.
Resultados — A temperatura, a duração do armazenamento e o diluente tiveram efeitos independentes na estabilidade química do CAZ. As concentrações de CAZ sofreram diminuição com o tempo e com o aumento da temperatura. As soluções NA+CAZ exibiram a menor degradação em comparação com as outras soluções. O MIC para PA foi mais consistente para soluções NA+CAZ, independentemente da temperatura de armazenamento e da duração do armazenamento.
Conclusões e importância clínica — A estabilidade química e microbiológica das soluções manipuladas de CAZ variou de acordo com o diluente, a temperatura de armazenamento e a duração do armazenamento. A diluição em NA resultou no menor variação na estabilidade ao longo de 28 dias em comparação com outros diluentes se armazenadas em temperaturas refrigeradas ou congeladas.

© 2021 The Authors. Veterinary Dermatology published by John Wiley & Sons Ltd on behalf of the European Society of Veterinary Dermatology and the American College of Veterinary Dermatology.