Stability of Colistin Methanesulfonate in Pharmaceutical Products and Solutions for Administration to Patients

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Colistin methanesulfonate (CMS) has the potential to hydrolyze in aqueous solution to liberate colistin, its microbiologically active and more toxic parent compound. While conversion of CMS to colistin in vivo is important for bactericidal activity, liberation of colistin during storage and/or use of pharmaceutical formulations may potentiate the toxicity of CMS. To date, there has been no information available regarding the stability of CMS in pharmaceutical preparations. Two commercial CMS formulations were investigated for stability with respect to colistin content, which was measured by a specific high-performance liquid chromatography method. Coly-Mycin M Parenteral (colistimethate lyophilized powder) was stable (<0.1% of CMS present as colistin) for at least 20 weeks at 4°C and 25°C at 60% relative humidity. When Coly-Mycin M was reconstituted with 2 ml of water to a CMS concentration of 200 mg/ml for injection, Coly-Mycin M was stable (<0.1% colistin formed) for at least 7 days at both 4°C and 25°C. When further diluted to 4 mg/ml in a glucose (5%) or saline (0.9%) infusion solution as directed, CMS hydrolyzed faster at 25°C (<4% colistin formed after 48 h) than at 4°C (0.3% colistin formed). The second formulation, CMS Solution for Inhalation (77.5 mg/ml), was stable at 4°C and 25°C for at least 12 months, as determined based on colistin content (<0.1%). This study demonstrated the concentration- and temperature-dependent hydrolysis of CMS. The information provided by this study has important implications for the formulation and clinical use of CMS products.

Colistin (also known as polymyxin E) (Fig. 1a) is a cationic peptide antibiotic used for treatment of infections caused by gram-negative bacteria such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae where other therapeutic options are ineffective. Colistin methanesulfonate (CMS; also known as colistimethate), the inactive prodrug form of colistin (3) (Fig. 1b), is the version of colistin most commonly used clinically. It is administered intravenously or intramuscularly, both of which are approved routes of administration (Colomycin Injection package insert, Forest Laboratories Ltd., Bexley, Kent, United Kingdom, 2006; Coly-Mycin M [colistimethate] Parenteral package insert, Monarch Pharmaceuticals, Bristol, TN, 2005; Coly-Mycin M Parenteral package insert, Pfizer Pty. Ltd., West Ryde, New South Wales, Australia, 2005). Other parenteral treatment routes, such as the intraventricular route, are also being used to treat difficult-to-manage infections (10, 15, 29). For many years, solutions of CMS have been administered by nebulization into the lungs of patients with cystic fibrosis (CF) to manage colonization or infections caused by P. aeruginosa (4, 5, 6, 14). CMS is also formulated for administration (Colomycin Injection package insert, Forest Laboratories Ltd., Bexley, Kent, United Kingdom, 2006; Coly-Mycin M Parenteral package insert, Pfizer Pty. Ltd., West Ryde, New South Wales, Australia, 2005). Other parenteral treatment routes, such as the intraventricular route, are also being used to treat difficult-to-manage infections (10, 15, 29). For many years, solutions of CMS have been administered by nebulization into the lungs of patients with cystic fibrosis (CF) to manage colonization or infections caused by P. aeruginosa (4, 5, 6, 14). CMS is also being increasingly used via inhalation to treat infections of patients with ventilator-associated pneumonia caused by multidrug-resistant gram-negative bacteria (12, 24, 27). Administration of CMS via inhalation is not approved by the FDA (30) but is common in CF clinics and intensive-care units throughout the world (9, 12–14, 27). The solution that is administered via nebulization to the lungs is either a solution made by reconstitution of the lyophilized parenteral formulation or a solution prepared by a hospital pharmacy department or other manufacturer specifically for inhalation that contains CMS at a concentration in the range of 40 to 200 mg/ml.

CMS is the form of colistin most commonly used clinically, as it is less nephro- and neurotoxic than colistin itself (33) and is less irritating to the airways following inhalation (1, 7, 32). In order for antimicrobial activity to occur, CMS must hydrolyze to liberate colistin (3). While the in vivo hydrolysis of CMS is required for an antibiotic effect, hydrolysis of CMS in vitro (e.g., during storage or use of pharmaceutical formulations) may have implications for toxicity. Recently, the purported formation of colistin in a solution of CMS (reconstituted from the CMS parenteral formulation Coly-Mycin M) was implicated in the death of a CF patient following inhalation (26) and this led to the issuing of an FDA alert (11). This unfortunate case highlights the need to elucidate the stability of CMS formulations, with respect to colistin content, to ensure that CMS formulations are used safely.

In dilute solutions (20 μg/ml to 100 μg/ml) in buffer and plasma, CMS has been shown to hydrolyze rapidly to produce colistin at 37°C (17). The extent of conversion of CMS to colistin in CMS pharmaceutical formulations under relevant conditions of storage and use has not been reported. Thus, the aim of the present study was to determine, under clinically relevant conditions, the extent of conversion of CMS to colistin...
in a commercial lyophilized product prepared for parenteral administration and the resultant solutions prepared from the powder for administration to patients and in a nebulization solution intended for delivery into the lungs.

MATERIALS AND METHODS

Chemicals. The colistin sulfate used in the study was a USP reference standard obtained from U.S. Pharmacopoeia (Rockville, MD). CMS sodium (batch 2780-04) was made by Alpharma (Copenhagen, Denmark). Acetone (high-performance liquid chromatography [HPLC] grade) was from Mallinkrodt Chemicals (Phillipsburg, NJ). All HPLC chemicals were of analytical reagent grade or equivalent. Boric acid, sodium hydroxide, 9-fluorenylmethyl chloroformate (FMOC-Cl), and ammonium nitrate were from Sigma Aldrich (Castle Hill, New South Wales, Australia). Water for Injections (WFI) was manufactured by Pharmacia and Upjohn (Bentley, West Australia, Australia). Water from the Millipore Milli-Q water system (Milford, MA). Sodium hydrogen carbonate was from Merck (FMOC Cl), and ammonium nitrate were from Sigma Aldrich (Castle Hill, New South Wales, Australia), and sodium hydroxide was from Merck Chemicals (Kilsyth, Victoria, Australia). Water for Injections (WFI) was manufactured by Pharmacia and Upjohn (Bentley, West Australia, Australia) in accordance with the British Pharmacopoeia standard. Sterile solutions of sodium chloride (0.9%) and glucose (5.0%) in 100-ml Viabags were produced by Baxter (Old Toongabbie, New South Wales, Australia). Water was purified by a Millipore Milli-Q water system (Milford, MA). CMS pharmaceutical products. Two pharmaceutical products of CMS were examined. A commercial parenteral CMS preparation, Coly-Mycin M Parenteral (batch 00564P1 manufactured May 2004; expiration date, May 2007), was from Pfizer Pty. Ltd. (West Ryde, Australia). The stability study commenced in May 2005. Vials contained lyophilized powder and were labeled as containing 150 mg of CMS sodium for inhalation (1,000,000 U/ml) (equivalent to 0.1% of the total CMS content having

RESULTS

The content of colistin present in the lyophilized powder of CMS sodium contained within vials of Coly-Mycin M Parenteral is plotted as a function of storage time in Fig. 2a. The amount of colistin present was very low (≤0.1% of CMS content) at both 4°C and 25°C over the 20-week period investigated. When the lyophilized powder of CMS contained within vials of Coly-Mycin M Parenteral was reconstituted in 2 ml of WFI, less than 0.1% of the original CMS content was again present as colistin in the reconstituted solution over the 7-day study period; this was the case for both 4°C and 25°C (Fig. 2b). The effect of diluting the reconstituted solution in 0.9% sodium chloride or 5.0% glucose on the formation of colistin is shown in Fig. 2c. Colistin was formed faster at 25°C than at 4°C, with approximately 4% of the total CMS content having converted to colistin after 48 h in both normal saline and

FIG. 1. Chemical structures of colistin (a) and CMS (b). The fatty acid is 6-methyloctanoic acid for colistin A and CMS A. The fatty acid is 6-methyloctanepropanoic acid for colistin B and CMS B. Thr, threonine; Leu, leucine; Dab, α,γ-diaminobutyric acid (α and γ indicate the respective primary amine groups involved in the peptide link).
glucose solutions at 25°C in comparison with approximately 0.3% at 4°C.

The content of colistin present in the WCH Solution for Inhalation across the 52-week study period is shown in Fig. 3. Less than approximately 0.1% of the CMS content was present as colistin throughout the 1-year period for solutions stored at either 4°C or 25°C (Fig. 3). The pH and osmolality of the inhalation solution did not change throughout the study period at either 4°C (pH, 6.93 ± 0.05; osmolality, 292 ± 11 mosmole) or 25°C (pH, 6.94 ± 0.06; osmolality, 294 ± 14 mosmole).

DISCUSSION

While the hydrolysis of CMS to colistin after administration of CMS to patients is essential for antibacterial activity, colistin formed in pharmaceutical preparations of CMS prior to administration may cause toxicity in patients. There has been no information available from use of specific analytical methods to date regarding the stability of CMS in pharmaceutical products. The recent death of a patient in a case in which colistin formed by hydrolysis in a CMS formulation was implicated (11, 26) highlights the importance of understanding the stability of CMS in pharmaceutical products.

In this study, Coly-Mycin M Parenteral lyophilized powder was shown to be stable (0.1% colistin) under the tested conditions for 20 weeks (Fig. 2a). The study was not designed to assess the stability of Coly-Mycin M Parenteral over its entire shelf life of 3 years. As conditions of storage prior to the commencement of the study were unknown, this study was intended to assess the stability of Coly-Mycin M Parenteral only within a defined period (prior to the expiration date) under known and controlled conditions of temperature and humidity.

Reconstitution of Coly-Mycin M Parenteral with 2 ml of WFI produced a solution with a nominal CMS concentration of 200 mg/ml. Interestingly, this solution was stable (0.1% colistin) for 7 days at 4°C and 25°C (Fig. 2b). The similar percentages of colistin in the lyophilized powder and in the reconstituted solution suggest that there was no formation of colistin over 7 days in the solution or only minimal formation. While this finding may indicate that this solution could be stored for up to 7 days prior to use, good clinical practice would dictate that the solution should be used as soon as possible. If it were necessary to store the solution for a brief period, it would be prudent to store it at 4°C to minimize bacterial contamination.

The product information insert for Coly-Mycin M Parenteral indicates that once reconstituted with 2 ml of WFI, the resulting solution should be diluted in one of several infusion fluids for administration to patients (Colomycin Injection package insert, Forest Laboratories Ltd., Bexley, Kent, United Kingdom, 2006; Coly-Mycin M Parenteral package insert,
Monarch Pharmaceuticals, Bristol, TN, 2005; Coly-Mycin M Parenteral package insert, Pfizer Pty. Ltd., West Ryde, New South Wales, Australia, 2005). After dilution, the nominal CMS concentration in the infusion solutions was 4 mg/ml. Over 48 h there was clear evidence of hydrolysis of CMS in the infusion solutions stored at 25°C (at 48 h there was <4% colistin present). The extent of colistin formation was substantially lower in the solutions stored at 4°C (<0.3% colistin at 48 h) (Fig. 2c). The practical significance of the stability data for the use of infusion solutions is that these solutions should be administered immediately after preparation. If any delay in administration is anticipated, infusion solutions should be stored at 4°C, as the formation of colistin is substantially reduced at this temperature. CMS is commonly administered intermittently as a short-term infusion (~30-min infusion duration) (21; Coly-Mycin M Parenteral package insert, Monarch Pharmaceuticals, Bristol, TN, 2005; Coly-Mycin M Parenteral package insert, Pfizer Pty. Ltd., West Ryde, New South Wales, Australia, 2005), but there have been some reports of administration of CMS by continuous intravenous infusion (8, 28). Infusion solutions left at room temperature are likely to contain 2 to 3% colistin after 12 to 24 h (Fig. 2c). It is difficult to speculate on the implications of the administration of infusion solutions containing these levels of colistin, since there is no reported clinical experience regarding intravenous administration of colistin (sulfate) in humans.

Arguably, the most remarkable results in this stability study were those obtained with the WCH Solution for Inhalation. Although this particular preparation is available only in Australia, preparations with similar CMS concentrations have been extensively used for inhalation therapy in Europe for more than 2 decades (25). Across the 52-week study period, this solution, containing 77.5 mg of CMS/ml and used at either 4°C or 25°C, was found to contain less than 0.1% of the original CMS content in the form of colistin (Fig. 3) while maintaining constant levels of pH and osmolality. Hydrolysis of CMS to colistin (via partially sulfomethylated derivatives of colistin) would be expected to be accompanied by a corresponding increase in the number of osmotically active species in the solution. That no change in osmolality occurred supports our finding determined from HPLC analysis showing minimal hydrolysis of CMS to colistin.

Inhalation of colistin (sulfate) is known to be irritating to the airways, causing bronchial hyperreactivity (32). Fewer problems with bronchoconstriction occur when CMS is administered by inhalation, but airway irritability can be variable (2, 7), possibly arising from the presence of colistin formed in vivo from CMS deposited in the airways. Recently, it was suggested that the death of a patient after inhalation of a premixed CMS solution may have been the result of the presence in the solution of colistin formed by hydrolysis of CMS (11, 26). In view of the scarcity of information available relating to that most unfortunate death, including a lack of details concerning the concentration of CMS in the solution and the transportation and storage conditions for the solution, it is difficult to extrapolate from the current stability findings to those pertaining to that event. However, the WCH Solution for Inhalation that was investigated in the present study yielded less than 0.1% colistin over a 1-year period when stored at either 4°C or 25°C.

Generally, the results from our present and previous studies indicate that the hydrolysis of CMS to colistin is both temperature and concentration dependent (3, 17). The temperature dependence of hydrolysis, as clearly evident in the results seen with the CMS infusion solutions (Fig. 2c), is perhaps not surprising. The concentration dependence is evident from the greater rates and extents of formation of colistin in dilute solutions of CMS (4 mg/ml in the infusion solutions) in comparison to those seen with CMS solutions of high concentrations (200 mg/ml and 77.5 mg/ml in reconstituted Coly-Mycin M Parenteral and WCH Solution for Inhalation, respectively).

In our previous studies, albeit conducted at 37°C, 60 to 80% of CMS was converted to colistin after 24 to 48 h in buffer and plasma at CMS concentrations of 0.1 mg/ml and 20 µg/ml, respectively (17), and after 4 h, approximately 30% of 20 µg/ml of CMS/ml was hydrolyzed to colistin in microbiological media (3). At clinically relevant plasma concentrations of CMS (10 to 20 µg/ml), rapid conversion to colistin in vivo has been demonstrated, with appreciable concentrations of colistin detected in plasma shortly after the administration of CMS in humans (16, 22) and rats (19, 31). The mechanism for the concentration-dependent hydrolysis of CMS to colistin is not understood and is the subject of an ongoing investigation. These data highlight the importance of conducting stability studies across a range of conditions, including those that are clinically relevant.

The HPLC analytical method used in the present study allows discrimination of colistin only from CMS and/or its partially sulfomethylated derivatives. It is possible that hydrolysis of CMS to partial derivatives occurred in the solutions studied, but these intermediates could not be quantified, and this is a recognized limitation of this study. However, as noted above, the fact that no change in the osmolality of the nebulization solution occurred over a 1-year period is consistent with minimal formation of partially sulfomethylated derivatives of CMS. It should also be noted that there is no published method currently available for measurement of partially sulfomethylated derivatives.

In conclusion, an understanding of the extent of colistin formation in CMS pharmaceutical products is essential for a clearer interpretation of results from preclinical and clinical studies. Such knowledge will aid appropriate routine clinical use of these products and should minimize the occurrence of adverse events associated with CMS use.

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