Colistin Methanesulfonate Is an Inactive Prodrug of Colistin against *Pseudomonas aeruginosa*

Phillip J. Bergen, Jian Li, Craig R. Rayner,† Roger L. Nation*

Facility for Anti-Infective Drug Development and Innovation, Victorian College of Pharmacy, Monash University, Parkville, Victoria, Australia

There is a dearth of information on the pharmacodynamics of "colistin," despite its increasing use as a last line of defense for treatment of infections caused by multidrug-resistant gram-negative organisms. The antimicrobial activities of colistin and colistin methanesulfonate (CMS) were investigated by studying the time-kill kinetics of each against a type culture of *Pseudomonas aeruginosa* in cation-adjusted Mueller-Hinton broth. The appearance of colistin from CMS spiked at 8.0 and 32 mg/liter was measured by high-performance liquid chromatography, which generated colistin concentration-time profiles. These concentration-time profiles were subsequently mimicked in other incubations, independent of CMS, by incrementally spiking colistin. When the cultures were spiked with CMS at either concentration, there was a substantial delay in the onset of the killing effect which was not evident until the concentrations of colistin generated from the hydrolysis of CMS had reached approximately 0.5 to 1 mg/liter (i.e., ~0.5 to 1 times the MIC for colistin). The time course of the killing effect was similar when colistin was added incrementally to achieve the same colistin concentration-time course observed from the hydrolysis of CMS. Given that the killing kinetics of CMS can be accounted for by the appearance of colistin, CMS is an inactive prodrug of colistin with activity against *P. aeruginosa*. This is the first study to demonstrate the formation of colistin in microbiological media containing CMS and to demonstrate that CMS is an inactive prodrug of colistin. These findings have important implications for susceptibility testing involving "colistin," in particular, for MIC measurement and for microbiological assays and pharmacokinetic and pharmacodynamic studies.

Globally there is a growing threat from the emergence of multidrug-resistant (MDR) microorganisms (7, 15, 18, 19). Although the threat from MDR gram-positive organisms has lessened, at least temporarily, owing to the development of new antimicrobial agents active against these organisms (36), the situation is quite different for MDR gram-negative bacteria (36, 37). With few new antibiotic classes in the drug development pipeline for the treatment of infections caused by MDR gram-negative bacteria, *Pseudomonas aeruginosa* in particular, we are unlikely to see any new advances in the treatment of infections caused by these organisms in the next few years. Unfortunately, MDR *P. aeruginosa* is increasing in prevalence (19, 35, 48); and infections with this organism are causing major clinical problems in patients with burns, neutropenia, or cystic fibrosis and in those who are immunocompromised (14, 47, 48). Several institutions have already experienced outbreaks of *P. aeruginosa* or *Acinetobacter baumannii* infections resistant to all commercially available antibiotics except the polymyxins (3, 5, 39, 46). It is precisely this scenario to which the Infectious Disease Society of America refers in its "Bad Bugs, No Drugs" campaign (18). Given these circumstances, a review of the activities and clinical use of many older antimicrobial drugs is occurring. With their rapid bactericidal activities and current low levels of resistance (6, 12, 16, 34), the polymyxins, and colistin (also known as polymyxin E) in particular, have undergone a revival as agents for treatment of infections caused by MDR *P. aeruginosa*, *A. baumannii*, and *Klebsiella pneumoniae* (6, 20, 30). Previously relegated to the status of a reserve agent after early reports of a "high" incidence of toxicity (21, 44), colistin is now a last line of defense for the treatment of infections with MDR gram-negative organisms (3, 30, 33).

Colistin is a cationic, multicomponent lipopeptide consisting of a cyclic heptapeptide with a tripeptide side chain acylated at the N terminus by a fatty acid (Fig. 1a). The two major components are colistin A (polymyxin E$_1$) and colistin B (polymyxin E$_2$) (41). Two different forms of colistin are available commercially: colistin sulfate (hereafter referred to as colistin) and sodium colistin methanesulfonate (CMS) (Fig. 1b). CMS is produced by the reaction of colistin with formaldehyde and sodium bisulfite (1), which leads to the addition of a sulfomethyl group to the primary amines of colistin. Colistin is primarily used topically, whereas CMS is used parenterally; both forms may be given by inhalation (30). CMS is less toxic than colistin when it is administered parenterally (4, 45), and indeed, this was the reason for the development of CMS. In aqueous solutions CMS undergoes hydrolysis to form a complex mixture of partially sulfomethylated derivatives, as well as colistin (1, 4, 25). By the use of high-performance liquid chromatographic (HPLC) methods for separate quantification of CMS and colistin in biological fluids (26, 27, 29), the formation of colistin in vivo has been demonstrated in patients (24, 31) and rats (28) receiving parenteral CMS; both the CMS administered and the colistin generated circulate in plasma. Further-

---

* Corresponding author. Mailing address: Facility for Anti-Infective Drug Development and Innovation, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia. Phone: 61 3 9903 9061. Fax: 61 3 9903 9629. E-mail: Roger .Nation@vcp.monash.edu.au.
† Present address: Roche Products Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, England.
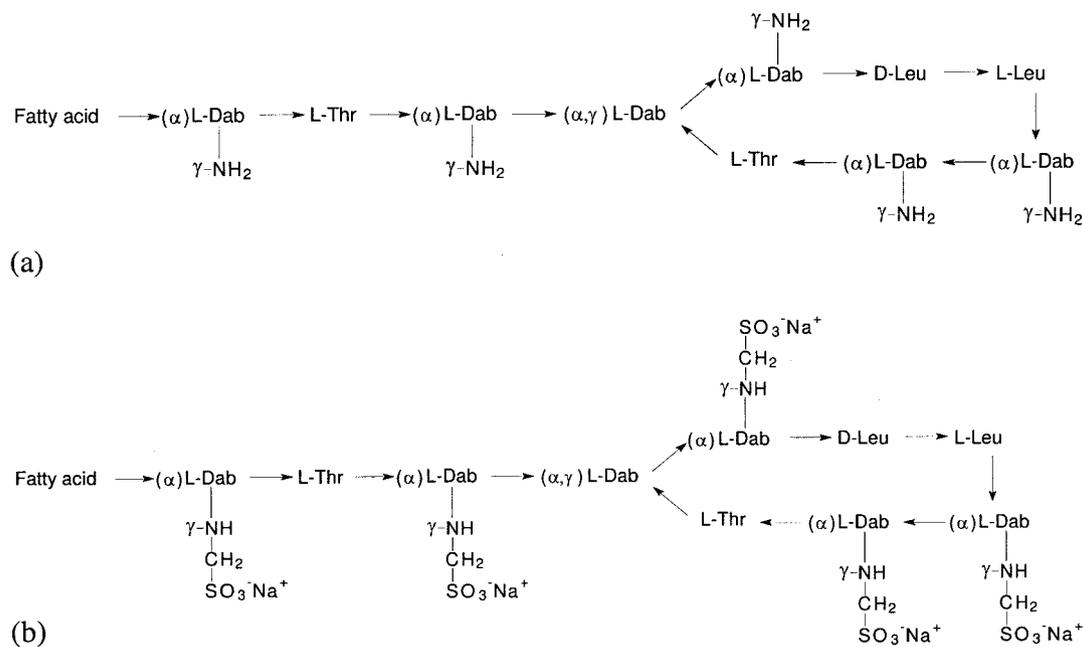
FIG. 1. (a) Structures of colistin A and B; (b) structures of sodium colistin A and B methanesulfonate (CMS). Fatty acid, 6-methyloctanoic acid for colistin A and 6-methylheptanoic acid for colistin B; Thr, threonine; Leu, leucine; Dab, α,γ-diaminobutyric acid. α and γ indicate the respective —NH₂ involved in the peptide linkage.

more, the pharmacokinetics of CMS and colistin have been demonstrated to differ (24, 28, 31).

Even after adjustment for molecular weight differences, CMS has reduced antibacterial activity compared with that of colistin, as assessed by MICs (2, 4, 11, 32, 45), and has been reported to be two to four times less active against *P. aeruginosa* (11, 32, 45). However, to our knowledge, no previous publications have reported on the contribution to bacterial killing made by each of CMS and colistin, the latter being formed via hydrolysis from CMS. Knowledge of the relative activities of CMS and colistin has important implications for standardization of susceptibility studies (e.g., MIC measurement), as well as microbiological assays of "colistin" in biological fluids; pharmacokinetic and pharmacodynamic studies involving "colistin" would also be affected. The aim of this study was therefore to determine the relative contributions of colistin and CMS to activities against *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains. A reference strain of *P. aeruginosa* ATCC 27853 (American Type Culture Collection, Manassas, VA) was used in this study. The strain was stored at −80°C in a cryovial storage container (Simport Plastics, Quebec, Canada). Fresh isolates were subcultured on horse blood agar (Media Preparation Unit, The University of Melbourne, Parkville, Australia) and incubated at 35°C for 24 h prior to each experiment. Calcium-adjusted Mueller-Hinton broth (CAMHB; lot 332998; Oxoid, Hampshire, England) was used.

Chemicals and reagents. Colistin sulfate (lot 072K1656; 19,530 units/mg) was purchased from Sigma-Aldrich (St. Louis, MO); 200-mg/liter and 500-mg/liter stock solutions were prepared in water and stored at 4°C before use. Colistin is stable under these conditions (25). Sodium colistin methanesulfonate (lot A1680552; 13,100 units/mg) was purchased from Alpharma Pharmaceuticals (Copenhagen, Denmark); stock solutions of 1,000 mg/liter in water were freshly prepared before each experiment to minimize the potential hydrolysis of CMS in aqueous solutions (25). All stock solutions were filtered by using 0.22-μm-pore-size Millex-GP filters (Millipore, Bedford, MA). All other chemicals were from the suppliers described previously (26).

Time-kill kinetics. The MIC of colistin (sulfate) and the apparent MIC of CMS for this strain, as determined by broth microdilution (8), were 1 and 4 mg/liter, respectively.

(i) Colistin methanesulfonate. Time-kill studies were conducted with two concentrations of CMS (8.0 and 32 mg/liter) that corresponded to two and eight times the apparent MIC of CMS against *P. aeruginosa* ATCC 27853, respectively. An aliquot (200 μl) of an overnight culture was added to 20 ml of CAMHB and incubated at 37°C until early log-phase growth was reached; 5 ml was then transferred to a 500-ml bottle (Schott Duran, Germany) containing 385 ml of CAMHB, which gave approximately 5 × 10⁵ CFU/ml. CMS was added to achieve an initial concentration of either 8.0 or 32 mg/liter. The experiment was conducted for 240 min in a shaking water bath (100 rpm) at 37°C. Serial samples were obtained at 0, 30, 60, 75, 90, 120, 135, 150, 165, 180, 210, and 240 min (2 ml per sample) for viable cell counting and determination of colistin concentrations and, where relevant, CMS concentrations (see below). Viable cell counting was conducted by spiral plating (WASP2 spiral plater; Don Whitley Scientific Ltd., England) 50 μl of appropriately diluted sample onto nutrient agar plates (Media Preparation Unit), followed by incubation at 35°C for 18 to 24 h. The colonies were counted with a ProtoCOL colony counter (Don Whitley Scientific Ltd.); the limit of detection was 20 CFU/ml. For each CMS concentration, three replicates were performed, each on a separate day. The time course of the concentrations of colistin formed by hydrolysis from CMS at each concentration were used as the basis for time-kill experiments involving colistin, as described below.

(ii) Colistin. The media and inoculum were prepared as described above for CMS. After the inoculation, colistin (sulfate) solution was added at 5-min intervals to mimic the concentration-time course of colistin produced by hydrolysis of CMS at 8.0 or 32 mg/liter, determined as described above (corrections were made to allow for differences in molecular weights between the base and the sulfate forms of colistin). Aliquots from either the 200-mg/liter or 500-mg/liter colistin solutions were used. The experiment was conducted for 240 min in a shaking water bath (100 rpm) at 37°C. Serial samples were obtained at 0, 30, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, and 240 min (2 ml per sample) for viable cell counting and determination of colistin concentrations. Three replicates were performed at each concentration, each on a separate day. In a control experiment, colistin was added to achieve an initial concentration of 6.0 mg/liter (six times the MIC of colistin). Serial samples were obtained at 0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min for viable cell counting (see above).
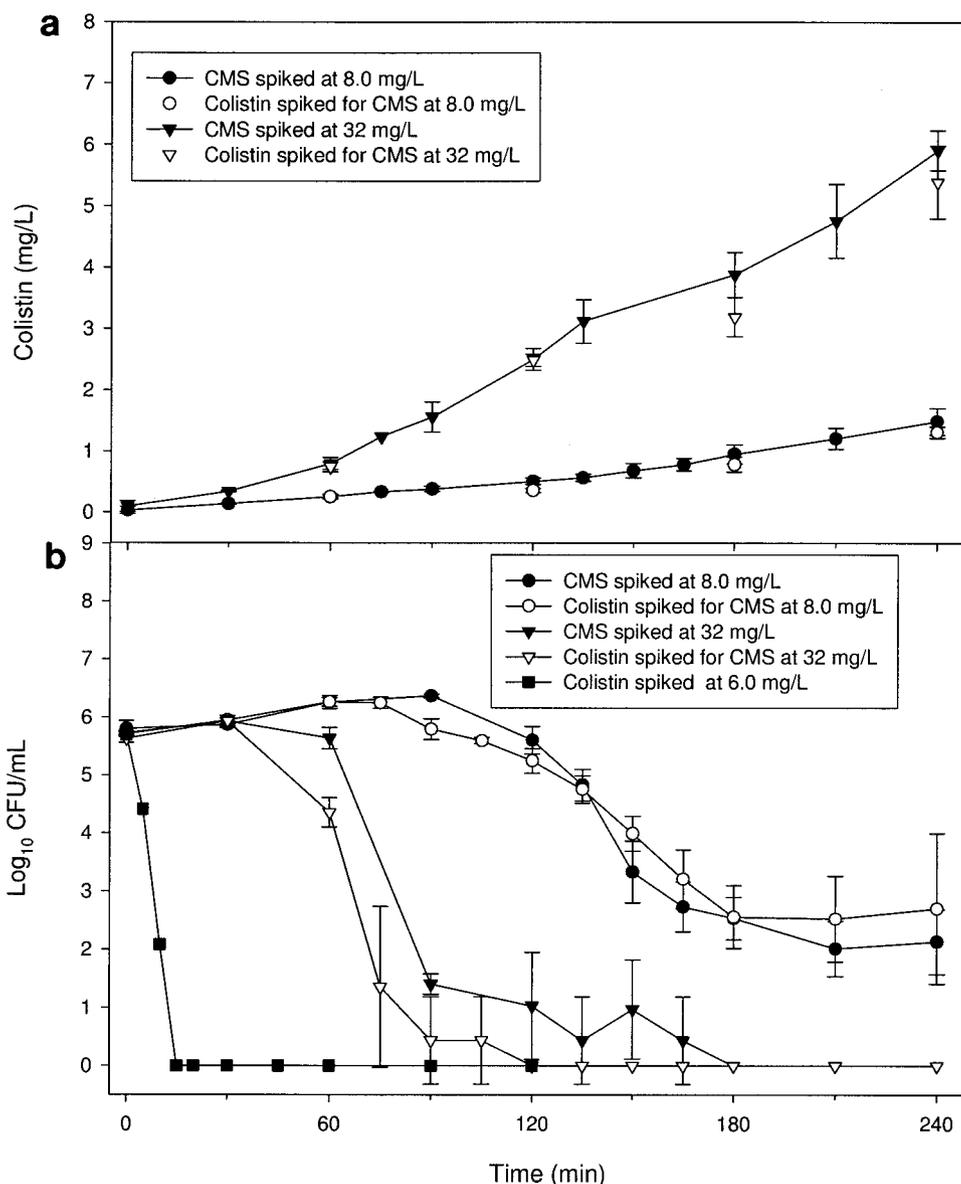
FIG. 2. (a) Concentration-time course of colistin produced from CMS spiked at 8.0 mg/liter and 32 mg/liter ($n = 3$) and from incremental spiking with colistin ($n = 3$); (b) time-kill curves for *P. aeruginosa* obtained by using colistin methanesulfonate spiked at 8.0 mg/liter and 32 mg/liter ($n = 3$) at zero time, spiked incrementally with colistin to mimic the colistin concentration-time course achieved after spiking of the sample with colistin methanesulfonate at 8.0 mg/liter and 32 mg/liter ($n = 3$), and colistin spiked at 6.0 mg/liter at zero time ($n = 1$; control experiment).

Determination of colistin and CMS in CAMHB. Samples from the time-kill studies were taken in duplicate (250 μl for colistin and 150 μl for CMS) and placed in 1.5-ml microcentrifuge tubes (Neptune; CLP, Mexico) and immediately stored at −80°C. The concentrations of colistin and CMS were measured by two sensitive HPLC methods previously developed by our group, with minor modifications (26, 27, 29). The assay ranges were 0.083 mg/liter to 5.80 mg/liter for colistin (base) and 0.25 mg/liter to 40 mg/liter for CMS. The accuracy and reproducibility of the results for the quality control samples for both assays fell within 10% of the target values.

Data analysis. The killing effects from addition of CMS or colistin were examined descriptively and were quantified by calculation of the area under the concentration-time curve of $\log_{10}$ CFU/ml from 0 to 240 min (AUC_{0-240}) normalized by $\log_{10}$ CFU/ml$_{t = 0}$, where $\log_{10}$ CFU/ml$_{t = 0}$ is the initial $\log_{10}$ CFU/ml value; this was not performed for the control experiment, where colistin was added at 6.0 mg/liter at zero time and samples were collected for 120 min. AUC_{0-240} was calculated by using the linear trapezoidal rule.

RESULTS

Time course of colistin formation from CMS. The initial CMS concentrations achieved were 7.64 ± 0.64 mg/liter ($n = 3$) for 8.0 mg/liter (two times the apparent MIC of CMS) and 29.0 ± 1.98 mg/liter ($n = 3$) for 32 mg/liter (eight times the apparent MIC of CMS). Figure 2a shows the mean colistin concentrations present after the samples were spiked with CMS. The concentrations of colistin present immediately following addition of CMS at 8.0 mg/liter were below the limit of quantification of 0.083 mg/liter for two of three samples and 0.10 mg/liter for the remaining sample; the concentrations present immediately following addition of CMS at 32 mg/liter

TABLE 1. AUC_{0-240} of killing curves normalized by
baseline $\log_{10} CFU/ml_{t = 0}$

Colistin form	$AUC_{0-240}/(\log_{10} CFU/ml_{t = 0})$ $(n = 3)$	
	8.0 mg/liter	32 mg/liter
CMS	186.3 ± 6.0	90.4 ± 4.1
Colistin[a]	192.8 ± 10.4	70.0 ± 7.4

[a] Spiked incrementally to achieve the same concentration-time course of colistin observed from hydrolysis of CMS spiked initially at 8.0 mg/liter or 32 mg/liter.

were 0.17 and 0.12 mg/liter for two of three samples and below the limit of quantification for the remaining sample. This indicates that the amount of colistin present in the batch of CMS used was very low. Following addition of CMS, comparatively small amounts of colistin were formed in the first 60 min of incubation: 0.26 ± 0.04 mg/liter from 8.0-mg/liter CMS and 0.80 ± 0.10 mg/liter from 32-mg/liter CMS. After 240 min of incubation, these values had risen to 1.49 ± 0.22 mg/liter (~1.5 times the MIC of colistin) from 8.0-mg/liter CMS and 5.91 ± 0.32 mg/liter (~6 times the MIC of colistin) from 32-mg/liter CMS; this corresponds (in molar terms) to 29.1% ± 2.1% and 30.5% ± 2.2%, respectively, of the CMS being converted to colistin. The concentration-time profiles of colistin achieved by using incremental spiking of colistin matched closely those derived from the hydrolysis of CMS in CAMHB (Fig. 2a).

Time-kill kinetics. The time-kill profiles achieved with CMS and colistin at each concentration are shown in Fig. 2b. In the control experiment in which colistin was spiked at 6.0 mg/liter (six times the MIC of colistin), no viable bacteria were detectable after 15 min. When CMS was spiked at 8.0 mg/liter, killing began at approximately 90 min when the concentration of colistin formed by hydrolysis of CMS was approximately 0.5 the MIC of colistin (0.46 ± 0.05 mg/liter of colistin). When CMS was spiked at 32 mg/liter, killing began at approximately 30 min, with a rapid decline in CFU/ml at 60 min, when the concentration of colistin formed by hydrolysis of CMS was approximately 1.0 the MIC of colistin (0.96 ± 0.12 mg/liter of colistin). In both cases, very similar time-kill profiles occurred when colistin was added incrementally to achieve the colistin concentration-time courses observed from the hydrolysis of CMS spiked initially with either 8.0 or 32 mg/liter CMS. The mean AUC_{0-240} values normalized by initial $\log_{10} CFU/ml$, $AUC_{0-240}/(\log_{10} CFU/ml_{t = 0})$, are shown in Table 1.

DISCUSSION

"Colistin" is now a last line of defense against infections caused by MDR gram-negative organisms (3, 30, 33, 35). Despite renewed interest in the clinical use of "colistin", confusion has surrounded its use in susceptibility studies, in particular MIC measurement, as well as in microbiological assays used to measure "colistin" concentrations in biological fluids. Information on the pharmacokinetics and pharmacodynamics of CMS and colistin, especially in critically ill patients, is also lacking (31, 38). Study of the antibacterial activity of CMS, the parenteral form of colistin, has proven complicated due to the hydrolytic conversion of CMS to colistin (25); and this is reflected in the literature (30). Prior to the present study, the

relative contributions of CMS and colistin to antibacterial activity have, to our knowledge, never been directly investigated.

The concentrations of CMS chosen for the present study (8.0 and 32 mg/liter) represent the concentrations achievable in plasma in vivo (24, 31). A control experiment in which colistin was spiked at 6.0 mg/liter (six times the MIC of colistin), equivalent to the concentration of colistin generated in 240 min after spiking of the samples with CMS at 32 mg/liter (Fig. 2a), demonstrated rapid and extensive killing such that the number of CFU/ml had fallen below the limit of detection of counting of viable organisms by 15 min (Fig. 2b); this is consistent with the findings of our previous report (32). In sharp contrast, when CMS was spiked at zero time to achieve concentrations equivalent to two and eight times the apparent MIC for CMS, there was a substantial delay in the onset of the killing effect (Fig. 2b); in both cases, growth continued for some time after addition of CMS (~75 min for CMS spiked at 8.0 mg/liter and ~30 min for CMS spiked at 32 mg/liter). This is significant, given that a concentration of CMS even eight times its apparent MIC was unable to cause bacterial killing until significant amounts of colistin had formed. As has already been shown with concentration-dependent anti-infectives, including colistin, bacterial killing occurs at concentrations below the MIC (32, 40). At both CMS concentrations, killing was not evident until the concentrations of colistin generated from the hydrolysis of CMS had reached approximately 0.5 to 1 mg/liter (i.e., ~0.5 to 1 times the MIC for colistin).

Importantly, the time course of the killing effect achieved by spiking with CMS at zero time (8.0 mg/liter or 32 mg/liter) was very similar to that observed when colistin was added incrementally to achieve the same colistin concentration-time course from the hydrolysis of CMS (Fig. 2). In particular, the early parts of the killing curves generated from CMS are virtually superimposable on the respective curves from incremental spiking of colistin; this is a time when very little colistin has been generated from CMS. Given that killing did not begin until significant amounts of colistin had formed, as mentioned above, it appears that CMS and the partially sulfomethylated derivatives possess little, if any, antibacterial activity. For each concentration of CMS (8.0 and 32 mg/liter), the $AUC_{0-240}/(\log_{10} CFU/ml_{t = 0})$ obtained by spiking of the samples with CMS or spiking of the samples incrementally with colistin to achieve the same colistin concentration-time course (Table 1) were within 3.5% and 22.5%, respectively. Consequently, the time course of antibacterial activity from CMS can be accounted for by the appearance of colistin. Thus, our study has clearly demonstrated that at both concentrations of CMS (8.0 and 32 mg/liter), antipseudomonal activity was due to the formation of colistin; CMS alone displayed no antibacterial activity. CMS may therefore be regarded as an inactive prodrug of colistin.

The present study has demonstrated that the formation of colistin in vivo following administration of CMS is a prerequisite for antibacterial activity. It is also clear that the in vitro hydrolysis of CMS to colistin during microbiological procedures conducted in the laboratory (e.g., MIC measurement) has the potential to make CMS appear to possess antibacterial activity. Although we recently reported for the first time colistin formation from CMS in vitro (25) and in vivo (24, 28, 31), to our knowledge no previous work has demonstrated the

formation of colistin in microbiological media spiked with CMS during incubation. In the present study, approximately 30% of the CMS present was hydrolyzed to colistin after only 240 min. In microbiological procedures such as MIC measurement, where similar temperature conditions but significantly longer incubation periods are used (up to 24 h for MIC measurement), even more colistin would be expected to form. This, together with our demonstration that CMS possesses no (or little) antibacterial activity, has very important implications for microbiological testing procedures.

Due to uncertainties over whether CMS possesses antibacterial activity in its own right, MIC measurements for "colistin" have been performed by using colistin (8) or CMS (6), or both (11, 32). This has caused confusion for clinicians and clinical microbiologists, as evidenced by discussions on the American Society for Microbiology e-mail discussion group (ClinMicroNet, 17 May 2005), with questions such as the relevance of an MIC test performed with colistin for a patient receiving CMS remaining unanswered. The present study has demonstrated that the use of CMS is inappropriate for MIC measurement, as antimicrobial activity is due to the formation of colistin and not to the CMS itself. Recently the Clinical and Laboratory Standards Institute published MIC measurement protocols and stated that colistin, not CMS, should be used when MICs are determined (8). This decision appears to be justified by our results. Given the emergence of resistance to colistin (32) and its increasing use, accurate susceptibility data will be vital for the meaningful inclusion of "colistin" in the testing lists of antimicrobial susceptibility studies; currently, "colistin" is seldom included in such studies (43).

The demonstration that colistin forms in microbiological media spiked with CMS also has important implications for measurement of "colistin" concentrations in biological fluids by microbiological assays. Current recommendations for the microbiological assay of "colistin" involve diffusion methods that use long periods of incubation at elevated temperatures (up to 24 h at 37°C) (23, 50), and significant amounts of colistin are likely to form via hydrolysis from CMS under these conditions. After parenteral administration of CMS, in vivo hydrolysis ensures that both CMS and colistin will be present at the time of collection of a blood sample (24, 28, 31). Over the duration of a microbiological assay, however, significant amounts of colistin will continue to form. Regardless of whether CMS or colistin is chosen as the reference standard against which biological samples from patients are compared, such assays are unable to differentiate between the colistin present at the time of sampling and the colistin formed via the hydrolysis of CMS during incubation. Consequently, microbiological assays of samples collected from patients administered CMS give no information about the individual concentrations of colistin and CMS present at the time of collection.

The inability to accurately determine colistin and CMS concentrations in biological fluids by analytical methods such as microbiological assay, as described above, has significant implications for past and future research on the pharmacokinetics and pharmacodynamics of "colistin." Many of the data on the pharmacokinetics of "colistin" that have been generated were obtained by microbiological assays (9, 13, 52). Some pharmacokinetic studies used HPLC methods that are potentially more specific for measurement of "colistin" concentrations in

humans (22, 42); however, these particular HPLC methods suffer from problems similar to those encountered when microbiological assays are used. In particular, such HPLC methods (42) cannot differentiate between the colistin present at the time of sampling and the colistin formed by hydrolysis subsequent to sample collection. Thus, most pharmacokinetic data on "colistin" published to date can be considered representative of a complex mixture of colistin and CMS and its partially sulfomethylated derivatives. Given that colistin formation from CMS occurs in vivo (24, 31) and, as demonstrated in the present study, that CMS is an inactive prodrug, future studies attempting to define pharmacokinetic and pharmacodynamic parameters must rely on assays that are capable of distinguishing between colistin and CMS, such as the HPLC methods described previously (26, 27).

An explanation as to why CMS displays little, if any, antimicrobial activity may reside in the postulated mechanism of action of the cationic peptides, which includes the polymyxins (16). For gram-negative microorganisms, it is proposed that the antibacterial activity of polymyxins involves a two-step process that begins with the displacement of divalent cations (Ca^{2+}, Mg^{2+}) on cell surface lipopolysaccharides of the outer membrane, followed by interaction with the negatively charged cytoplasmic membrane (16); the increasing net positive charge of the peptide promotes interaction with each membrane (17, 51). Given that at physiological pH the net charge on CMS is -5, the charges on its partially sulfomethylated derivatives range from -3 to $+3$ (-3 with four attached sulfomethyl groups, $+3$ with one attached sulfomethyl group), whereas the net charge on colistin is $+5$, the strongly cationic colistin would be expected to have greater antibacterial activity. While we acknowledge both the limitations of the present study to draw definitive conclusions on this issue and the fact that structural parameters other than charge are important for antibacterial activity in most peptides (10, 17, 49, 51), the postulated mechanism of action of the cationic peptides combined with the polycationic nature of colistin may explain why CMS acts only as a prodrug.

In conclusion, we have demonstrated the formation of colistin during incubation in microbiological media spiked with CMS by sensitive and specific HPLC methods for the quantification of colistin and CMS. Using a reference strain of *P. aeruginosa*, we investigated the contribution to antimicrobial activity of CMS and its hydrolysis product, colistin, which forms in solution from CMS. Our conclusion that CMS is an inactive prodrug of colistin, as well as our demonstration that colistin forms from CMS in microbiological media, has important implications for susceptibility testing involving "colistin," in particular, for MIC measurement, as well as for microbiological assays and pharmacokinetic and pharmacodynamic studies.

REFERENCES

1. **Barnett, M., S. R. Bushby, and S. Wilkinson.** 1964. Sodium sulphomethyl derivatives of polymyxins. Br. J. Pharmacol. **23:**552–574.

2. **Bergan, T., and J. Fuglesang.** 1982. Polymyxin antibiotics: chemical and pharmacokinetic properties. Antibiot. Chemother. **31:**119–144.

3. **Berlana, D., J. M. Llop, E. Fort, M. B. Badia, and R. Jodar.** 2005. Use of colistin in the treatment of multiple-drug-resistant gram-negative infections. Am. J. Health-Syst. Pharm. **62:**39–47.

4. **Beveridge, E. G., and A. J. Martin.** 1967. Sodium sulphomethyl derivatives of polymyxins. Br. J. Pharmacol. Chemother. **29:**125–135.

5. **Boutiba-Ben Boubaker, I., J. Boukadida, O. Triki, N. Hannachi, and S. Ben Redjeb.** 2003. Outbreak of nosocomial urinary tract infections due to a multidrug resistant Pseudomonas aeruginosa. Pathol. Biol. **51:**147–150.

6. **Catchpole, C. R., J. M. Andrews, N. Brenwald, and R. Wise.** 1997. A reassessment of the in-vitro activity of colistin sulphomethate sodium. J. Antimicrob. Chemother. **39:**255–260.

7. **Chastre, J., and J. L. Trouillet.** 2000. Problem pathogens (Pseudomonas aeruginosa and Acinetobacter). Semin. Respir. Infect. **15:**287–298.

8. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing; 15th information supplement (M100-S15). Clinical and Laboratory Standards Institute, Wayne, Pa.

9. **Conway, S. P., M. N. Pond, A. Watson, C. Etherington, H. L. Robey, and M. H. Goldman.** 1997. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. Thorax **52:**987–993.

10. **Dathe, M., T. Wieprecht, H. Nikolenko, L. Handel, W. L. Maloy, D. L. MacDonald, M. Beyermann, and M. Bienert.** 1997. Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides. FEBS Lett. **403:**208–212.

11. **Eickhoff, T. C., and M. Finland.** 1965. Polymyxin B and colistin: in vitro activity against Pseudomonas aeruginosa. Am. J. Med. Sci. **249:**172–174.

12. **Evans, M. E., D. J. Feola, and R. P. Rapp.** 1999. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. Ann. Pharmacother. **33:**960–967.

13. **Froman, J., L. Gross, and S. Curatola.** 1970. Serum and urine levels following parenteral administration of sodium colistimethate to normal individuals. J. Urol. **103:**210–214.

14. **Giamarellou, H., and A. Antoniadou.** 2001. Antipseudomonal antibiotics. Med. Clin. N. Am. **85:**19–42.

15. **Hanberger, H., D. Diekema, A. Fluit, R. Jones, M. Struelens, R. Spencer, and M. Wolff.** 2001. Surveillance of antibiotic resistance in European ICUs. J. Hosp. Infect. **48:**161–176.

16. **Hancock, R. E., and D. S. Chapple.** 1999. Peptide antibiotics. Antimicrob. Agents Chemother. **43:**1317–1323.

17. **Hancock, R. E., T. Falla, and M. Brown.** 1995. Cationic bactericidal peptides. Adv. Microb. Physiol. **37:**135–175.

18. **Infectious Diseases Society of America.** 2004, posting date. Bad bugs, no drugs. Infectious Diseases Society of America, Alexandria, Va. [Online.] http://www.idsociety.org/pa/IDSA_Paper4_final_web.pdf.

19. **Jones, R. N.** 2001. Resistance patterns among nosocomial pathogens: trends over the past few years. Chest **119:**397S–404S.

20. **Karabinis, A., E. Paramythiotou, D. Mylona-Petropoulou, A. Kalogeromitros, N. Katsarelis, F. Kontopidou, I. Poularas, and H. Malamou-Lada.** 2004. Colistin for Klebsiella pneumoniae-associated sepsis. Clin. Infect. Dis. **38:**e7–e9.

21. **Koch-Weser, J., V. W. Sidel, E. B. Federman, P. Kanarek, D. C. Finer, and A. E. Eaton.** 1970. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. Ann. Intern. Med. **72:**857–868.

22. **Le Brun, P. P., A. I. de Graaf, and A. A. Vinks.** 2000. High-performance liquid chromatographic method for the determination of colistin in serum. Ther. Drug Monit. **22:**589–593.

23. **Leroy, P., D. Decolin, S. Nicolas, P. Archimbault, and A. Nicolas.** 1989. Residue determination of two co-administered antibacterial agents—cephalexin and colistin—in calf tissues using high-performance liquid chromatography and microbiological methods. J. Pharm. Biomed. Anal. **7:**1837–1846.

24. **Li, J., K. Coulthard, R. Milne, R. L. Nation, S. Conway, D. Peckham, C. Etherington, and J. Turnidge.** 2003. Steady-state pharmacokinetics of intravenous colistin methanesulphonate in patients with cystic fibrosis. J. Antimicrob. Chemother. **52:**987–992.

25. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, and K. Coulthard.** 2003. Stability of colistin and colistin methanesulfonate in aqueous media and plasma as determined by high-performance liquid chromatography. Antimicrob. Agents Chemother. **47:**1364–1370.

26. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, K. Coulthard, and D. W. Johnson.** 2001. A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. **761:**167–175.

27. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, K. Coulthard, and J. Valentine.** 2002. Simple method for assaying colistin methanesulfonate in plasma and urine using high-performance liquid chromatography. Antimicrob. Agents Chemother. **46:**3304–3307.

28. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, T. C. Smeaton, and K. Coulthard.** 2004. Pharmacokinetics of colistin methanesulphonate and colistin in rats following an intravenous dose of colistin methanesulphonate. J. Antimicrob. Chemother. **53:**837–840.

29. **Li, J., R. W. Milne, R. L. Nation, J. D. Turnidge, T. C. Smeaton, and K. Coulthard.** 2003. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. Antimicrob. Agents Chemother. **47:**1766–1770.

30. **Li, J., R. L. Nation, R. W. Milne, J. D. Turnidge, and K. Coulthard.** 2005. Evaluation of colistin as an agent against multi-resistant gram-negative bacteria. Int. J. Antimicrob. Agents **25:**11–25.

31. **Li, J., C. R. Rayner, R. L. Nation, R. Deans, R. Boots, N. Widdecombe, A. Douglas, and J. Lipman.** 2005. Pharmacokinetics of colistin methanesulfonate and colistin in a critically ill patient receiving continuous venovenous hemodiafiltration. Antimicrob. Agents Chemother. **49:**4814–4815.

32. **Li, J., J. Turnidge, R. Milne, R. L. Nation, and K. Coulthard.** 2001. In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother. **45:**781–785.

33. **Linden, P. K., S. Kusne, K. Coley, P. Fontes, D. J. Kramer, and D. Paterson.** 2003. Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant *Pseudomonas aeruginosa*. Clin. Infect. Dis. **37:**e154–e160.

34. **Littlewood, J. M., C. Koch, P. A. Lambert, N. Hoiby, J. S. Elborn, S. P. Conway, R. Dinwiddie, and F. Duncan-Skingle.** 2000. A ten year review of colomycin. Respir. Med. **94:**632–640.

35. **Livermore, D. M.** 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa:* our worst nightmare? Clin. Infect. Dis. **34:**634–640.

36. **Livermore, D. M.** 2004. The need for new antibiotics. Clin. Microbiol. Infect. **10**(Suppl. 4):1–9.

37. **Livermore, D. M.** 2003. The threat from the pink corner. Ann. Med. **35:**226–234.

38. **Markou, N., H. Apostolakos, C. Koumoudiou, M. Athanasiou, A. Koutsoukou, I. Alamanos, and L. Gregorakos.** 2003. Intravenous colistin in the treatment of sepsis from multiresistant gram-negative bacilli in critically ill patients. Crit. Care **7:**R78–R83.

39. **Michalopoulos, A., S. K. Kasiakou, E. S. Rosmarakis, and M. E. Falagas.** 2005. Cure of multidrug-resistant Acinetobacter baumannii bacteraemia with continuous intravenous infusion of colistin. Scand. J. Infect. Dis. **37:**142–145.

40. **Mouton, J. W., and A. A. Vinks.** 2005. Pharmacokinetic/pharmacodynamic modelling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. Clin. Pharmacokinet. **44:**201–210.

41. **Orwa, J. A., C. Govaerts, R. Busson, E. Roets, A. Van Schepdael, and J. Hoogmartens.** 2001. Isolation and structural characterization of colistin components. J. Antibiot. (Tokyo) **54:**595–599.

42. **Reed, M. D., R. C. Stern, M. A. O'Riordan, and J. L. Blumer.** 2001. The pharmacokinetics of colistin in patients with cystic fibrosis. J. Clin. Pharmacol. **41:**645–654.

43. **Rossolini, G. M., and E. Mantengoli.** 2005. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. Clin. Microbiol. Infect. **11**(Suppl. 4):17–32.

44. **Ryan, K. J., L. I. Schainuck, R. O. Hickman, and G. E. Striker.** 1969. Colistimethate toxicity. Report of a fatal case in a previously healthy child. JAMA **207:**2099–2101.

45. **Schwartz, B. S., M. R. Warren, F. A. Barkley, and L. Landis.** 1959. Microbiological and pharmacological studies of colistin sulfate and sodium colistinmethanesulfonate. Antibiot. Annu. **7:**41–60.

46. **Stein, A., and D. Raoult.** 2002. Colistin: an antimicrobial for the 21st century? Clin. Infect. Dis. **35:**901–902.

47. **Tatterson, L. E., J. F. Poschet, A. Firoved, J. Skidmore, and V. Deretic.** 2001. CFTR and pseudomonas infections in cystic fibrosis. Front. Biosci. **6:**D890–D897.

48. **Waterer, G. W., and R. G. Wunderink.** 2001. Increasing threat of gram-negative bacteria. Crit. Care Med. **29:**N75–N81.

49. **Wieprecht, T., M. Dathe, E. Krause, M. Beyermann, W. L. Maloy, D. L. MacDonald, and M. Bienert.** 1997. Modulation of membrane activity of amphipathic, antibacterial peptides by slight modifications of the hydrophobic moment. FEBS Lett. **417:**135–140.

50. **Wootton, M., H. A. Holt, and A. P. Macgowan.** 2005. Development of a novel assay method for colistin sulphomethate. Clin. Microbiol. Infect. **11:**243–244.

51. **Wu, M., and R. E. Hancock.** 1999. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. J. Biol. Chem. **274:**29–35.

52. **Yamada, S., T. Mayahara, N. Mitsuhashi, K. Wakabayashi, and K. Hiratsuka.** 1974. Colistin sodium methanesulfonate (colimycin, CL-M). I. Blood level, distribution, and excretion of CL-M. Jpn. J. Antibiot. **27:**8–14.