Chapter 14
Pharmacokinetics and Pharmacodynamics of Colistin

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Abstract The increasing prevalence of multidrug-resistant Gram-negative bacteria worldwide has resulted in colistin, administered as its inactive prodrug colistin methanesulfonate (CMS), being increasingly used as a last-line therapy to treat infections caused by these pathogens. Developed well before contemporary drug development procedures, substantial improvements in the understanding of its chemistry, pharmacokinetics (PK), pharmacodynamics (PD) and PK/PD relationships have occurred over the last decade which have enabled substantial progress towards optimising its clinical use in different patient populations. This has resulted in the first scientifically based dosing algorithm for various categories of critically ill patients receiving CMS to generate a desired target steady-state plasma concentration of formed colistin. It has become clear that monotherapy with CMS is unlikely to generate plasma colistin concentrations that are reliably efficacious. With nephrotoxicity preventing simply increasing the dose of CMS, combination therapy may be required in order to maximise efficacy and minimise the emergence of resistance.

Keywords Colistin • Colistin methanesulfonate • Pharmacokinetics • Pharmacodynamics • Dose optimization • *P. aeruginosa* • *A. baumannii* • *K. pneumoniae* • Multidrug resistance

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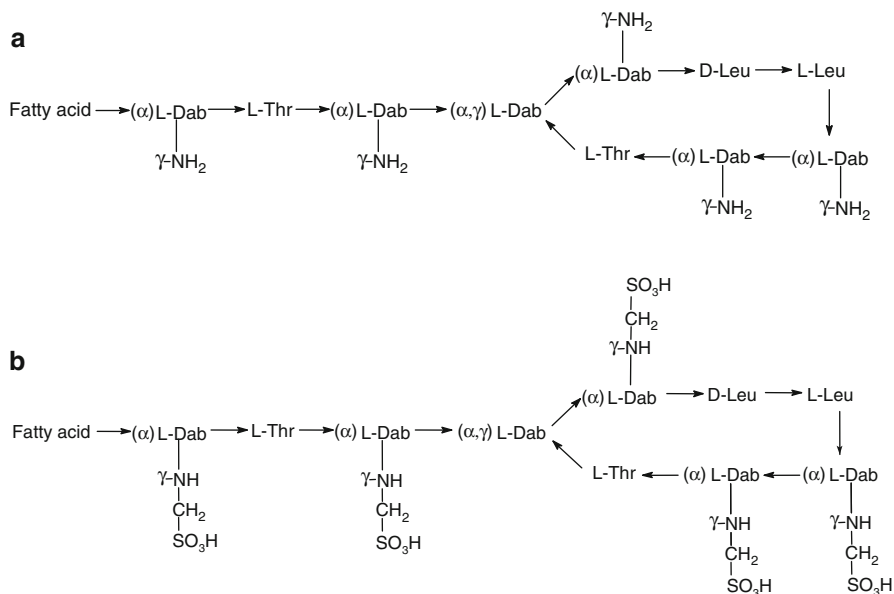e-mail: roger.nation@monash.edu

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Introduction

The increasing prevalence of infections caused by multidrug-resistant (MDR) Gram-negative bacteria, especially *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and the dearth of new antibiotics with activity against these pathogens reaching the clinic (Livermore 2004; Boucher et al. 2009; Opal and Calandra 2009; IDSA 2010; Spellberg et al. 2011), mean that physicians are needing to resort to use of the polymyxin class of antibiotics (Li et al. 2006b; Landman et al. 2008; Nation and Li 2009; Lim et al. 2010; Michalopoulos and Karatza 2010). These are 'old' antibiotics that were discovered in the late 1940s and then became available in the clinic about a decade later. While a number of polymyxins are known, only two (polymyxin B and E, the latter also known more commonly as colistin) are used clinically. Of the two polymyxins, colistin is most commonly used in the majority of places in the world and is the subject of this chapter.

Drug development and regulatory approval processes were much different in the 1950s than those existing today. As a result, colistin was never subjected to the scientific rigour required of modern pharmaceuticals before they become available for use in patients. It is not surprising, therefore, that there has been a major paucity of pharmacological and other scientific information that is needed to guide rational use of colistin in various categories of patients. Over the last decade or so, since the resurgence in its clinical use, there have been a small number of research groups conducting studies to establish the scientific basis for clinical utilisation of colistin. In essence this antibiotic, that is more than half a century old, has been subjected over the last few years to scientific investigation and evaluation that is akin to the drug development procedures required of newly discovered pharmaceuticals.

At the outset, it is important to indicate what will, and what will not, be reviewed in this chapter. The literature supporting, or otherwise, the clinical efficacy of colistin will not be examined. Not unexpectedly in view of the history surrounding colistin, there is a shortage of information relating to clinical efficacy. Most reports have been based upon retrospective studies and randomised controlled trials are conspicuous by their absence; those interested may consult other sources of information (Li et al. 2006b; Landman et al. 2008; Molina et al. 2009). Like all other drugs, use of colistin may be associated with adverse effects. The most worrying potential adverse effect of colistin is nephrotoxicity (Falagas and Kasiakou 2006; Hartzell et al. 2009; DeRyke et al. 2010; Kwon et al. 2010; Ko et al. 2011). Fortunately, if this adverse effect occurs, it is usually mild to moderate in nature and reversible upon discontinuation of colistin therapy (Falagas et al. 2005; Falagas and Kasiakou 2006; Betrosian et al. 2008; Pintado et al. 2008; Hartzell et al. 2009). Colistin-induced nephrotoxicity appears to involve accumulation of colistin in renal tubular cells mediated by transporters (Li et al. 2003c, 2004; Ma et al. 2009) and ensuing oxidative stress (Ozyilmaz et al. 2011; Yousef et al. 2011, 2012). Clinically used antioxidants, *N*-acetylcysteine, melatonin and ascorbic acid, have been shown in animal models to ameliorate colistin-induced nephrotoxicity (Ozyilmaz et al. 2011; Yousef et al. 2011, 2012) and they hold promise for application in patients.

a

Fatty acid → (α)L-Dab → L-Thr → (α)L-Dab → (α,γ) L-Dab

with γ-NH₂ branches rendered:

$$\text{Fatty acid} \rightarrow (\alpha)\text{L-Dab} \rightarrow \text{L-Thr} \rightarrow (\alpha)\text{L-Dab} \rightarrow (\alpha,\gamma)\text{L-Dab}$$

(α)L-Dab → D-Leu → L-Leu

γ-NH₂ (on (α)L-Dab)

L-Thr ← (α) L-Dab ← (α)L-Dab

γ-NH₂   γ-NH₂

γ-NH₂ (under first L-Dab), γ-NH₂ (under third L-Dab)

b

SO₃H
CH₂
γ-NH

Fatty acid → (α)L-Dab → L-Thr → (α) L-Dab → (α,γ) L-Dab

(α) L-Dab → D-Leu → L-Leu

L-Thr ← (α) L-Dab ← (α) L-Dab

γ-NH
CH₂
SO₃H (multiple branches)

Fig. 14.1 Structures of (**a**) colistin A and B and (**b**) colistin A and B methanesulfonate. 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 amino group involved in the peptide linkage

Nephrotoxicity will not be further reviewed here. This chapter will provide an overview of the current state of microbiological and pharmacological knowledge, especially in relation to the pharmacokinetic (PK) and pharmacodynamic (PD) properties of colistin, and the PK/PD driver of its antibacterial effect. Because an appreciation of the chemistry and related terminology (including that applied to the material used in the clinic) is essential to an understanding of the pharmacological and microbiological properties of colistin, there is no option but to briefly review these aspects.

Important Aspects of Chemistry

Colistin is a polypeptide antibiotic produced by *Bacillus colistinus* (Koyama et al. 1950). It comprises a heptapeptide ring with a tripeptide side chain to which is covalently linked a fatty acyl tail. It is a multicomponent antibiotic, with colistin A (polymyxin E1) and colistin B (polymyxin E2), which differ by only one carbon and two protons in the fatty acyl tail, being the two major components (Koyama et al. 1950; Orwa et al. 2000, 2001) (Fig. 14.1a). Because of the biological origin of this antibiotic, there is supplier-to-supplier and batch-to-batch variation in the proportion

of colistin A and colistin B in commercial material (Decolin et al. 1997; Li et al. 2001a). At physiological pH, the primary amines in the α,γ-diaminobutyric acid (Dab) residues (pKa approximately 10) are ionised; thus, colistin is a cationic antimicrobial peptide. Because colistin contains both polar (by virtue of the ionised Dab residues) and hydrophobic regions (fatty acyl tail), the molecule is amphiphilic and thereby exhibits surface activity (Wallace et al. 2010). As discussed below, both the cationic Dab residues and the hydrophobic fatty acyl tail are important for the interaction of colistin with the outer membrane of Gram-negative bacteria, a key first step in the bactericidal action of this antibiotic. Colistin is available commercially in the form of its colistin methanesulfonate salt. Colistin methanesulfonate is not administered parenterally, but is used in some countries in topical pharmaceutical formulations.

Colistin methanesulfonate (CMS, also known as colistimethate; Fig. 14.1b) is the form of 'colistin' that is administered parenterally and by inhalation. The sodium salt of CMS, in lyophilized form, is present in parenteral (and inhalational) formulations. It is crucial to understand the relationship between CMS and colistin. CMS was developed during the 1950s because of concerns in early studies about the relatively high level of toxicity that was associated with parenteral administration of colistin methanesulfonate. CMS is prepared from colistin by reaction of the free γ-amino groups of the five Dab residues with formaldehyde followed by sodium bisulfite (Barnett et al. 1964; Beveridge and Martin 1967). CMS is an inactive prodrug (Bergen et al. 2006) and is converted in vivo to the active antibacterial entity, colistin (Li et al. 2003a, 2004, 2005b; Markou et al. 2008; Plachouras et al. 2009; Imberti et al. 2010; Marchand et al. 2010a; Couet et al. 2011; Garonzik et al. 2011; Mohamed et al. 2012). It is essential to understand that the conversion of CMS to colistin, a requirement for antibacterial activity in vivo, may also occur in vitro as CMS is not stable in aqueous environments (Li et al. 2003b; Wallace et al. 2008b, 2010). Thus, the conversion of CMS to colistin, via a number of partially sulfomethylated derivatives, has been demonstrated to occur not only in vivo (see below) but also in vitro in plasma, urine, buffer solutions and microbiological culture medium (Li et al. 2003b, 2004; Bergen et al. 2006). The conversion of CMS to colistin also occurs in the solutions for administration to patients that are produced by reconstitution of the lyophilized powder in pharmaceutical products (Wallace et al. 2008b); to minimise this in vitro conversion, such reconstitution should occur immediately prior to administration. An awareness that CMS converts to colistin in aqueous media highlights the importance in PK and PK/PD studies of ensuring that blood, plasma and other biological samples are processed and stored appropriately to avoid in vitro conversion occurring after collection of samples (Dudhani et al. 2010a). Such conversion would result in a spuriously high estimate of the colistin concentration present in the sample at the time of its collection. Moreover, an appreciation of the facile conversion of CMS to colistin in aqueous media clearly leads to the conclusion that colistin methanesulfonate, rather than CMS sodium, must be used in determination of minimum inhibitory concentration (MIC). The use of CMS in MIC determinations will lead to an 'apparent' MIC that will represent the activity of the progressively increasing amount of colistin liberated from the CMS during the

course of the microbiological incubation (Bergen et al. 2006). The time course of liberation of colistin may vary from laboratory to laboratory dependent upon conditions employed, and this would be expected to lead to variability in MIC values for a given strain.

Antibacterial Properties

Spectrum of Activity

Colistin exhibits a narrow antibacterial spectrum of activity, mostly against common Gram-negative pathogens (Li et al. 2005a). Colistin retains excellent in vitro bactericidal activity against most common species of Gram-negative bacilli or coccobacilli including *P. aeruginosa* (Walkty et al. 2009; Cernohorska and Slavikova 2010; Gales et al. 2011), *Acinetobacter* spp. (Walkty et al. 2009; Yau et al. 2009; Gales et al. 2011; Queenan et al. 2012) and *Klebsiella* spp. (Walkty et al. 2009; Hawser 2010; Gales et al. 2011; Sader et al. 2011), the organisms against which it is most commonly used clinically. Activity against other Gram-negative bacterial species has been reviewed elsewhere (Falagas and Kasiakou 2005; Li et al. 2005a). Colistin has no significant activity against most Gram-positive bacteria (Schwartz et al. 1959; Finland et al. 1976a, b) or fungi (Hoeprich 1970).

Susceptibility Breakpoints

The breakpoints for colistin susceptibility are based on colistin methanesulfonate given that CMS is an inactive prodrug (Bergen et al. 2006), and different breakpoints have been employed by various organisations (Comite de l' Antibiogramme de la Societe Francaise de Microbiologie (SFM) 2005; Andrews and Howe 2011; Clinical and Laboratory Standards Institute (CLSI) 2012; European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2012). The Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints are ≤2 mg/L for both *P. aeruginosa* and *A. baumannii* using the microbroth dilution method (Clinical and Laboratory Standards Institute (CLSI) 2012). Given the emerging clinical pharmacokinetic and pharmacodynamic data (see sections below), the appropriateness of these breakpoints within a clinical context remains to be determined. Worryingly, colistin heteroresistance, the presence of resistant subpopulations within an isolate that is susceptible based upon its MIC, has been observed in *A. baumannii* (Li et al. 2006c; Owen et al. 2007; Tan et al. 2007; Hawley et al. 2008; Yau et al. 2009), *K. pneumoniae* (Poudyal et al. 2008; Meletis et al. 2011) and *P. aeruginosa* (Bergen et al. 2011a). While colistin still retains excellent activity generally, resistance to

colistin is increasing in several key species including *P. aeruginosa* (Johansen et al. 2008; Lee et al. 2011), *A. baumannii* (Ko et al. 2007; Al-Sweih et al. 2011), *K. pneumoniae* and other *Enterobacteriaceae* (Tan and Ng 2006; Kontopoulou et al. 2010; Suh et al. 2010; Toth et al. 2010; Bogdanovich et al. 2011; Marchaim et al. 2011; Mezzatesta et al. 2011), and *S. maltophilia* (Tan and Ng 2006).

Mechanisms of Activity and Resistance

When considering the mechanism of antimicrobial activity it must be remembered that the polymyxins are polycationic, amphiphilic peptides. As colistin and polymyxin B are structurally similar, differing by only one amino acid, they are believed to share the same mechanism of antibacterial action. The initial target of the polymyxins against Gram-negative bacteria is the lipopolysaccharide (LPS) component of the outer membrane, initiated by electrostatic attraction between the cationic polymyxin molecule and the anionic lipid A of LPS, thereby displacing divalent inorganic cations (Ca^{2+}, Mg^{2+}) that assist in stabilising the LPS leaflet (Hancock and Chapple 1999). Once electrostatically bound to LPS, the *N*-terminal fatty-acyl tail is inserted into the outer membrane in a process driven by hydrophobic interactions. The overall result is permeabilization of the outer membrane, allowing the polymyxin to access the periplasmic space and the cytoplasmic membrane; this is the so-called 'self-promoted uptake' mechanism (Hancock and Chapple 1999). Originally it was proposed that the polymyxin inserted into the cytoplasmic membrane forming conductance events leading to leakage of cell contents and cell death (Hancock et al. 1995). However, there is an increasing body of evidence that suggests the polymyxins exert their effects through an alternative mode of action or that they may in fact act upon multiple bacterial cell targets (Hancock and Rozek 2002; Brogden 2005; Hale and Hancock 2007). The exact mechanism(s) by which they ultimately kill bacterial cells is still unknown.

Given that the crucial first step in the action of polymyxins on Gram-negative bacterial cells is the electrostatic interaction between the positively charged polymyxins and the negatively charged LPS, it is not surprising that resistance to polymyxins often involves changes in LPS structure which decrease the negative charge on the cell surface and hence the electrostatic interactions with the peptide. Modifications to the lipid A and/or core of LPS typically mask phosphate groups with moieties such as aminoarabinose and phosphoethanolamine. Such modifications have been observed in *P. aeruginosa* (Moskowitz et al. 2004), *K. pneumoniae* (Helander et al. 1996) and other bacterial species (Morrison and Wenzel 1984; Breazeale et al. 2005; Winfield et al. 2005) and have been shown to increase resistance to polymyxins (Moskowitz et al. 2004; Breazeale et al. 2005; Lewis et al. 2009; Beceiro et al. 2011). Interestingly, it was recently shown that resistance in *A. baumannii* can be mediated by complete loss of LPS (Moffatt et al. 2010). In *K. pneumoniae*, the presence of capsule may also be important for polymyxin resistance (Helander et al. 1996; Llobet et al. 2008).

Inconsistent Labelling and Dose Regimens of Pharmaceutical Products

Unfortunately, different conventions are used in various parts of the world for labelling the content of CMS pharmaceutical products and in defining the recommended daily doses (Li et al. 2006a, b). In some parts of the world (e.g. Europe), CMS parenteral vials are labelled in international units (IU). In these countries there are usually three vial sizes containing 500,000 IU, 1 million IU and 2 million IU per vial, corresponding to approximately 40, 80 and 160 mg of CMS sodium per vial (since there are ~12,500 IU per mg of CMS sodium). In many countries in the world (e.g. USA, Canada, Australia), the parenteral product available is labelled in terms of 'colistin base activity'. In these countries, one vial size only is available and this contains 150 mg of colistin base activity, which actually corresponds to ~400 mg CMS sodium.

Very unfortunately, the inconsistency does not end with labelling of the pharmaceutical products; it extends to the recommended daily doses in the respective product information. For those products labelled in international units, the typical recommended dose for a patient over 60 kg and with normal renal function is 1–2 million IU three times daily (Li et al. 2006b), equivalent to 240–480 mg CMS sodium per day. For those products labelled in terms of colistin base activity, the recommended doses are 2.5–5 mg/kg colistin base activity per day in 2–4 divided doses (Li et al. 2006b), which is equivalent to about 6.67–13.3 mg/kg of CMS sodium per day. Thus, for a patient with normal renal function and bodyweight of 60 kg, the recommended dose of such a product labelled in terms of colistin base activity (recommended dose of 400–800 mg CMS sodium per day) is almost double that of the products that are labelled in international units (recommended dose of 240–480 mg CMS sodium per day, see above). The origin of this major discrepancy in recommended doses between products appears to be lost in the sands of time. Because both regimens appear to be equally well tolerated and because of the need to ensure the maximum antibacterial effect in an era of increasing multidrug resistance and shortage of new antibiotics, the higher of the two recommended dosage regimens (i.e. 2.5–5 mg/kg colistin base activity per day, equivalent to 400–800 mg CMS sodium per day) would seem to be a wise choice; as discussed below, a reduction in the daily dose may be appropriate for patients with impaired renal function.

The inconsistent labelling convention and the discrepant recommended dosage regimens have the potential to cause much confusion; indeed, even those clinicians who are familiar with the use of CMS are often unaware of this problem. Tragically, the confusion surrounding CMS labelling recently resulted in the death of a patient in the USA (Institute for Safe Medication Practices (ISMP) 2011). In that case, the physician ordered the dose as mg of CMS rather than as colistin base activity, the usual method of expressing the dose in the USA. This went unrecognised by the pharmacist and nurses and resulted in the patient receiving doses ~2.7-fold higher than intended. The patient subsequently developed acute renal failure and other complications that resulted in their death. Calls a number of years ago for an

international consensus (Li et al. 2006a) have not been actioned. Clinicians reading the international literature to inform their practice will need to remain vigilant in regard to interpretation of dosage regimens used in published studies.

Pharmacokinetics, Pharmacodynamics and Pharmacokinetic/ Pharmacodynamic Relationships

Important Methodological Considerations for PK, PD and PK/PD Studies

At the outset, it is essential to comment upon the analytical methods that have been employed in PK studies over the years. Such studies conducted through the 1950s to the 1990s inclusive were undertaken using microbiological assays (Mackay and Kaye 1964; al-Khayyat and Aronson 1973); indeed, such assays have been used in some of the more recent studies (Aoki et al. 2009). These assays, when applied to biological samples containing both CMS and colistin formed in vivo, are not capable of differentiating between the colistin actually present in the sample at the time of its collection from a subject administered CMS and the colistin formed in vitro during the incubation period of the microbiological assay. Thus, the use of such assays is incapable of providing accurate information on the time course of plasma concentrations of the prodrug (CMS) and the active entity (colistin). The pharmacokinetic characteristics described within the current prescribing information for the various parenteral products was obtained using microbiological assays; thus it is unhelpful and does not provide a solid scientific basis for understanding the disposition of administered CMS and the colistin formed from it in vivo.

An accurate and complete understanding of the PK of CMS and formed colistin has only been possible over the last 5–10 years since the development of HPLC (Li et al. 2001a, 2002) and LC/MS/MS (Jansson et al. 2009; Gobin et al. 2010; Dotsikas et al. 2011) analytical methods for the separate quantification of CMS and colistin in biological samples. Here, a couple of important points should be made. Firstly, all of the current methods for HPLC or LC/MS/MS analysis of 'CMS' involve so-called 'difference assays'. That is, the 'CMS' concentration in a biological sample is determined as the difference between the colistin concentration measured in a sample that has been carefully processed and stored to prevent in vitro conversion of CMS to colistin and the colistin concentration in another aliquot of the sample where the conversion of CMS to colistin is forced to occur in vitro. The 'CMS' concentration determined using this approach represents the concentration of CMS (i.e. the penta-sulfomethylated species) and the numerous partially sulfomethylated species that are intermediates in the conversion of CMS to colistin. This type of analytical approach has been necessary because it has not been possible to directly quantify CMS due to the complex chemical nature and composition of CMS. Secondly, it is essential to appreciate that very careful procedures must be employed

in the handling and storage of biological samples to avoid the in vitro conversion of CMS to colistin. Such in vitro conversion would lead to an underestimation of the 'CMS' concentration and, more importantly, an overestimation of the colistin concentration in the biological sample. Thus, upon their collection, samples must be placed on ice, processed rapidly (e.g. blood samples centrifuged to obtain plasma/serum) and stored under conditions to minimise in vitro conversion of CMS to colistin prior to analysis. In regard to the latter, it has been demonstrated that storage of plasma samples at −20 °C is generally not acceptable, unless the samples are analysed within 1 month of collection (Dudhani et al. 2010a). Samples should be stored at −70 °C to −80 °C and even then the samples must be analysed within 4 months of collection to avoid substantial conversion of CMS into colistin and the degradation of both entities.

Overview of the Pharmacokinetics of CMS and Formed Colistin

The availability in the past decade or so of liquid chromatographic methods has enabled increased understanding of the relatively complex disposition of the inactive prodrug, CMS and the (active) colistin formed from it in the body. The intravenous route is the most common way in which CMS is administered, especially in critically ill patients with life-threatening infections caused by Gram-negative bacteria. For this reason, and also because PK data obtained from studies using this route are the most informative in regard to dispositional characteristics, the major focus here will be on studies conducted following intravenous administration. This section will provide an overview of preclinical PK studies; this is important because there are aspects of the overall PK of CMS and formed colistin that are only possible to reveal by undertaking studies involving separate administration of CMS and preformed colistin, which cannot be readily performed in humans.

The differences in chemistry between CMS and colistin (see section entitled 'Important Aspects of Chemistry') translate into differences in the PK of these entities. Li et al. was the first to apply HPLC methods capable of distinguishing between CMS and colistin to studies undertaken in rats administered either CMS (Li et al. 2004) or colistin (Li et al. 2003c). Those studies provided very useful information concerning the differences in disposition of CMS and the colistin formed from it in vivo. Following intravenous administration of CMS in rats, colistin appeared in plasma soon after administration of the prodrug (Li et al. 2004). The terminal half-life of formed colistin was approximately twice that of the administered CMS and was similar to the half-life of colistin administered directly (Li et al. 2003c). This indicated that the overall disposition of formed colistin following administration of CMS was rate limited by its elimination rather than its formation. The fundamental aspects of the overall disposition of CMS and formed colistin observed by Li et al. (2004) were subsequently confirmed by Marchand et al. using a wide range of CMS doses (5–120 mg/kg intravenously) in a rat PK study (Marchand et al. 2010b). In people with cystic fibrosis, the terminal half-life of formed colistin (251 ± 79 min;

mean ± SD) has also been reported to be approximately twice that of the administered CMS (124 ± 52 min) (Li et al. 2003a). More recent studies conducted in critically ill patients indicated that the terminal half-life of formed colistin is substantially longer (up to ~18 h) than that of the CMS (~3 h) that was administered (Markou et al. 2008; Plachouras et al. 2009; Garonzik et al. 2011; Mohamed et al. 2012); it is also evident that the half-life of formed colistin in critically ill patients is longer than that in people with cystic fibrosis (Li et al. 2003a), which may relate to differences in renal function and other patient characteristics (see section 'How Appropriate Are Current Dosage Regimens?'). From studies conducted to date, the PK of CMS and formed colistin appears to be linear following intravenous administration of CMS. In rats administered intravenous CMS across the range 5–120 mg/kg (which generated plasma concentrations of CMS and formed colistin that span those that are clinically relevant) linear relationships were observed between CMS and colistin areas under the plasma concentration–time curves (AUC) to infinity and CMS doses, as well as between CMS and colistin maximum plasma concentration (C_{max}) values and CMS doses (Marchand et al. 2010b). Following direct administration of colistin subcutaneously across a range of colistin doses to infected neutropenic mice, there was evidence of non-linear PK (plasma colistin concentration increased to a greater extent than the increase in dose) (Dudhani et al. 2010b); however, this may have resulted from non-linearity in the tissue binding of colistin, including at the subcutaneous site of administration, thereby impacting the fraction of the dose available for absorption.

Studies performed several decades ago employing microbiological assays indicated that colistin binds extensively to tissues of many organs, whereas a lesser degree of tissue binding was apparent for CMS (Kunin and Bugg 1971; Craig and Kunin 1973; Ziv et al. 1982; Leroy et al. 1989). The studies with CMS must be interpreted cautiously due to the use of a microbiological assay, which is non-specific for CMS as the assay measures the concentration of active colistin generated from CMS in vivo as well as during the incubation period of the microbiological assay. Protein binding studies in plasma from a range of healthy (i.e. non-infected) animals indicated that colistin was 30–70 % plasma bound (Ziv and Sulman 1972; al-Khayyat and Aronson 1973; Li et al. 2003c). A recent study of colistin binding in plasma from infected neutropenic mice indicated that the binding of colistin was higher than that above (Dudhani et al. 2010b). It is apparent that colistin binds to both albumin and α_1-acid glycoprotein (Dudhani et al. 2009), the latter being an acute-phase reactant protein whose plasma concentration increases in a variety of stressful conditions, including infection (Voulgari et al. 1982; Morita and Yamaji 1995). The extent of plasma binding of colistin in infected patients may therefore be subject to variations in the concentrations of albumin, α_1-acid glycoprotein and any other proteins involved in its binding.

There are very substantial differences in the clearance pathways for CMS and colistin. Following intravenous administration of colistin in rats, substantially less than 1 % of the dose was recovered in urine in unchanged form (Li et al. 2003c); the renal clearance involved very extensive renal tubular reabsorption to an extent greater than that occurring for water indicating that the reabsorption of colistin must

be a carrier-mediated process (Li et al. 2003c; Ma et al. 2009). The very minor role for renal clearance in the overall body clearance of colistin was also observed for polymyxin B (differing from colistin in just one amino acid) in patients (Zavascki et al. 2008). In marked contrast, CMS was shown to be predominantly renally cleared in rats with a component of tubular secretion (Li et al. 2004). The trafficking through renal tubular cells of CMS by secretion [with the possibility of intracellular generation of colistin (Li et al. 2004)] and of colistin by tubular reabsorption (Li et al. 2003c) may explain in part the propensity for nephrotoxicity following administration of CMS. Comparison of the dose-normalised AUC of formed colistin arising from administration of CMS in rats with that arising from direct administration of colistin allowed estimation of the fraction of the dose of CMS that was converted systemically to colistin (Li et al. 2004); this revealed that only a very small proportion (~7 %) of the administered dose of CMS was converted to colistin. A subsequent study in rats by Marchand et al. (Marchand et al. 2010b) confirmed many of the observations of Li et al. (Li et al. 2004). Similar to the findings in rats, Couet et al. recently demonstrated that in young healthy volunteers administered a single dose of one million IU of CMS (infused over 1 h), CMS was predominantly excreted in the urine (70 % on average as both CMS and colistin, the majority of the latter forming in the urinary tract) (Couet et al. 2011). The low in vivo fractional conversion of the prodrug, CMS, to the active form, colistin, arises because the conversion clearance of CMS to colistin is substantially lower than the renal clearance of CMS (i.e. the fractional conversion is dictated by the relative efficiencies of parallel pathways for elimination of CMS).

As a result of the understanding generated from these studies, the overall disposition of CMS and formed colistin has been summarised as shown in Fig. 14.2. The schema shown in Fig. 14.2 is consistent with the emerging data on the pharmacokinetics of CMS and formed colistin in humans, which is discussed below (see section 'How Appropriate Are Current Dosage Regimens?').

Pharmacodynamics of Colistin

Although colistin is administered parenterally as CMS, it is important to recognise that antimicrobial activity results from formation of colistin, not from CMS or its partially sulfomethylated derivatives (Bergen et al. 2006). Thus, CMS should be considered an inactive prodrug of colistin. Most PD data on colistin have been generated using in vitro models. Time-kill studies with colistin (used as its sulfate salt) in both static and dynamic systems showed potent, concentration-dependent killing against *P. aeruginosa* (Eickhoff and Finland 1965; Li et al. 2001b; Gunderson et al. 2003; Bergen et al. 2008, 2010, 2011a, b; Bulitta et al. 2010; Lin et al. 2010)*, A. baumannii* (Owen et al. 2007; Tan et al. 2007) and *K. pneumoniae* (Poudyal et al. 2008; Deris et al. 2012), including multidrug-resistant and colistin-heteroresistant strains. Initial killing is very rapid, with a large decrease in colony-forming units (cfu) per mL occurring as early as 5 min after exposure to colistin concentrations in the vicinity
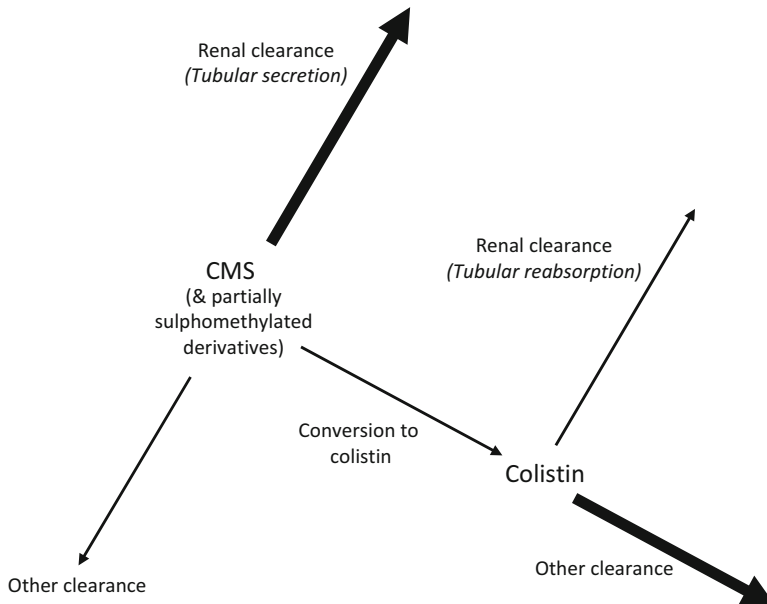
Fig. 14.2 Schematic representation of the disposition of colistin methanesulfonate and the colistin generated from it in the body following administration of sodium colistin methanesulfonate. Modified after (Li et al. 2006b) with permission from Elsevier

of the MIC and above. A modest post-antibiotic effect was found only at high colistin concentrations (Li et al. 2001b; Owen et al. 2007; Poudyal et al. 2008). Both the rate and extent of killing are markedly decreased at high compared to low inocula (Bulitta et al. 2010; Bergen et al. 2011a, b; Deris et al. 2012). Against a genetically characterised isolate of *P. aeruginosa* (PAO1), killing of the susceptible population by colistin was 23-fold slower at an inoculum of 10^9 cfu/mL and sixfold slower at 10^8 cfu/mL compared with 10^6 cfu/mL. Up to 32-fold higher colistin concentrations were required at the 10^9 compared with the 10^6 cfu/mL inoculum to achieve bactericidal activity (a ≥ 3-$\log_{10}$ cfu/mL decrease) (Bulitta et al. 2010). Thus, there is a potential need for higher colistin exposure or combination regimens to treat deep-seated, difficult-to-treat infections with high inocula.

A consistent finding of both in vitro and in vivo studies is regrowth with colistin monotherapy, even with concentrations well above those which can be safely achieved clinically. For example, two studies which utilised in vitro PD models (Gunderson et al. 2003; Bergen et al. 2010) reported regrowth of *P. aeruginosa* with colistin concentrations well above clinically achievable levels, the former with concentrations up to 200 mg/L, while similar regrowth has been reported in *A. baumannii* (Owen et al. 2007) and *K. pneumoniae* (Poudyal et al. 2008; Deris et al. 2012) with colistin concentrations up to 64 × MIC. Regrowth following colistin monotherapy has been shown to be the result of amplification of colistin-resistant sub-populations (Tan et al. 2007; Bergen et al. 2008, 2011a, b; Poudyal et al. 2008;

Bulitta et al. 2010; Dudhani et al. 2010b; Deris et al. 2012). The difficulty of eradicating colistin-resistant subpopulations with colistin monotherapy, together with the potential for rapid amplification of colistin-resistant subpopulations, suggests caution with the use of colistin monotherapy and highlights the importance of investigating rational colistin combinations.

Which Pharmacokinetic/Pharmacodynamic Index Is Most Predictive of Efficacy?

Only recently have studies used a dose-fractionation design to investigate the relationship between the PK and PD of colistin, namely which PK/PD index [C_{max}/MIC, AUC/MIC, or $T_{>MIC}$ (time for which concentrations exceed the MIC)] best correlates with colistin efficacy (Ketthireddy et al. 2007; Bergen et al. 2010; Dudhani et al. 2010b, c; Hengzhuang et al. 2012). Using an in vitro PK/PD model, Bergen et al. examined 37 different regimens involving various colistin C_{max} and dosage frequencies (including intermittent dosing and continuous infusion regimens) against three strains of *P. aeruginosa* including a colistin-susceptible but MDR strain (Bergen et al. 2010); analysis was based upon unbound or free (f) indices (i.e. fC_{max}/MIC, $fAUC$/MIC, and $fT_{>MIC}$). The overall killing effect was best correlated with $fAUC$/MIC ($R^2 = 0.931$); weaker correlations occurred for fC_{max}/MIC ($R^2 = 0.868$) and $fT_{>MIC}$ ($R^2 = 0.785$) (Fig. 14.3). The magnitudes of $fAUC$/MIC required for 1- and 2-$\log_{10}$ reductions in the area under the cfu/mL curve relative to growth control were able to be determined.

In a conference abstract describing use of a neutropenic mouse thigh infection model, Ketthireddy et al. concluded that once-daily dosing of colistin was most effective against *P. aeruginosa* and that C_{max}/MIC was likely the PK/PD index most predictive of efficacy; PK data, however, were not included in that study (Ketthireddy et al. 2007). Dudhani et al. employed neutropenic mouse thigh and lung infection models in dose-fractionation studies with colistin against three strains each of *P. aeruginosa* and *A. baumannii* which included MDR but colistin-susceptible and, for *A. baumannii*, colistin-heteroresistant strains (Dudhani et al. 2010b, c). In these studies, the time course of total (i.e. protein-bound plus unbound) and unbound plasma colistin concentrations were determined allowing the PK/PD analysis to be based upon unbound indices. The PK/PD index most predictive of the antibacterial effect against both *P. aeruginosa* and *A. baumannii* in both thigh and lung infection models was $fAUC$/MIC (see Fig. 14.4, for colistin against *P. aeruginosa* in murine lung infection model), in agreement with the results from dose-fractionation studies of colistin against *P. aeruginosa* in an in vitro PK/PD model (Bergen et al. 2010). That $fAUC$/MIC is the most predictive PK/PD index indicates that time-averaged exposure to colistin is more important than the achievement of high peak concentrations from the administration of larger, less frequent doses. For both *P. aeruginosa* and *A. baumannii*, $fAUC$/MIC targets required to achieve various magnitudes of
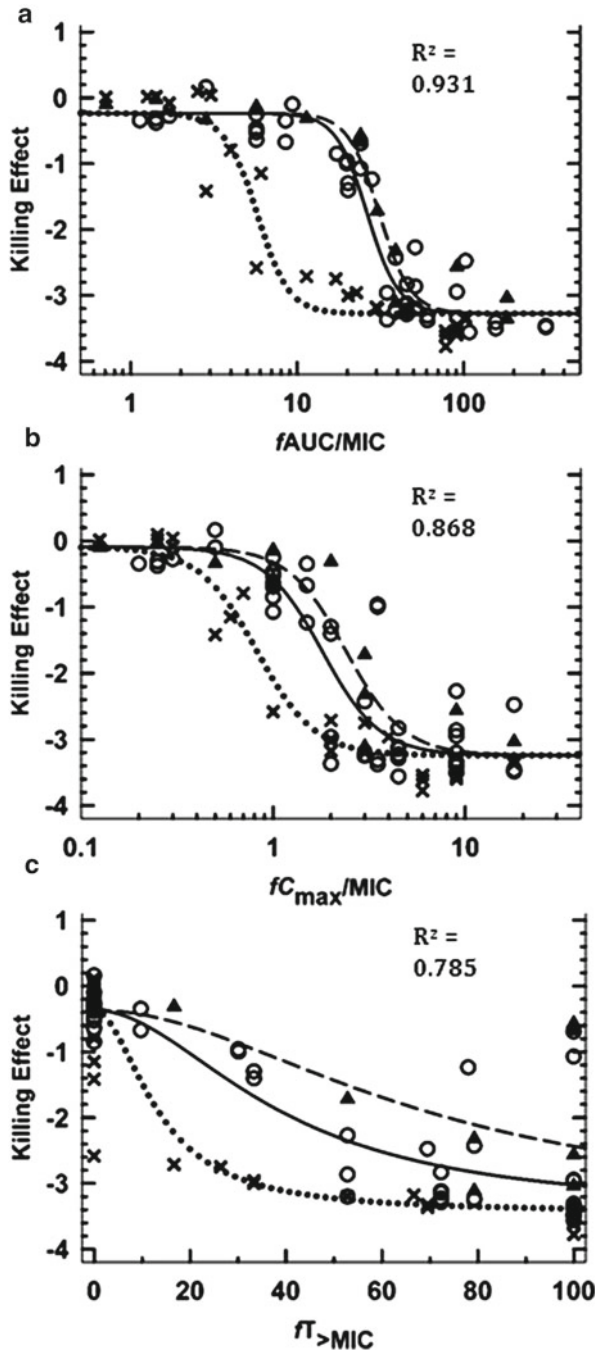
Fig. 14.3 Relationship between killing effect against three strains of *P. aeruginosa* as a function of three PK/PD indices: (**a**) fAUC/MIC, (**b**) fC_{max}/MIC and (**c**) $fT_{>MIC}$. ATCC 27853 (*solid line* and *open circles*), PAO1 (*dashed line* and *solid triangles*) and 19056 mucoid (*dotted line* and *crosses*). Each *data point* represents the result from a single treatment run. *Lines* represent model-generated fits. Reproduced from (Bergen et al. 2010) with permission from the American Society for Microbiology
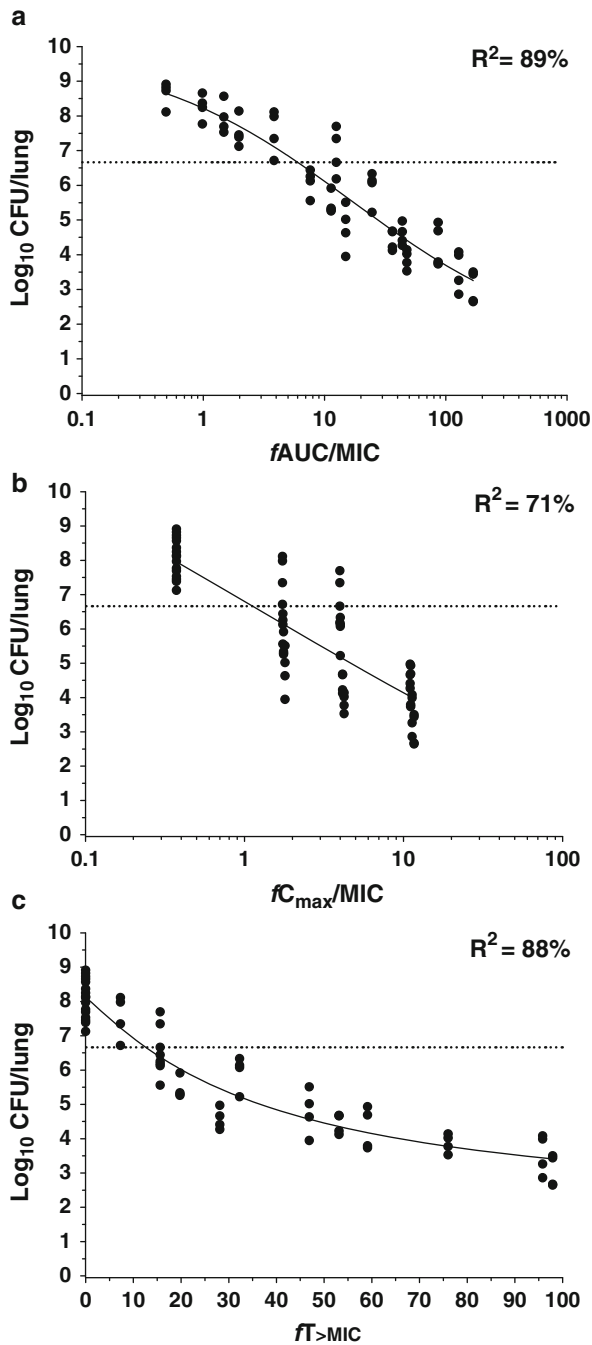
Fig. 14.4 Relationships for *P. aeruginosa* ATCC 27853 between the $\log_{10}$ CFU per lung at 24 h and the PK/PD indices (**a**) *f*AUC/MIC, (**b**) *f*C_{max}/MIC and (**c**) *f*T$_{>MIC}$. Each symbol represents the mean datum per mouse from two lungs. R^2 is the coefficient of determination. The *dotted line* represents the mean bacterial burden in lungs at the start of treatment. Reproduced from (Dudhani et al. 2010b, c) with permission from the American Society for Microbiology

kill were of the same order of magnitude in both the thigh and lung, although somewhat higher values were required to achieve maximal killing in the lung. Most recently, Hengzhuang et al. used a neutropenic murine lung biofilm infection model to determine the PK/PD indices for colistin most predictive of activity against planktonic and biofilm cells of a single strain of *P. aeruginosa* (Hengzhuang et al. 2012). The AUC/MIC was again the PK/PD index most closely correlated with bacterial killing of planktonic cells, whereas the AUC to minimal biofilm inhibitory concentration (MBIC) ratio (AUC/MBIC) was most predictive of killing for biofilm cells in the lung. In this study unbound colistin concentrations were not considered and AUC/MIC and AUC/MBIC values were those for total colistin. The AUC/MBIC targets identified to achieve various magnitudes of bacterial killing were substantially higher for biofilm infections than for planktonic cells. The observed differences in bacterial killing of planktonic and biofilm cells by Hengzhuang et al. (2012), as well as between thigh and lung infections by Dudhani et al. (2010b, c), suggest that dosage regimens may need to be altered depending upon the nature and/or site of infection.

Unfortunately, it is currently not possible to compare the fAUC/MIC targets identified from dose-fractionation studies in in vitro and animal infection models with the fAUC/MIC values achieved in infected patients receiving currently recommended CMS dosage regimens. Although, as discussed in the following section 'How Appropriate Are Current Dosage Regimens?', there is increasing information on the total plasma colistin concentrations occurring in CMS-treated patients, no information is currently available on unbound plasma concentrations. As such information is forthcoming it will be possible to not only assess the ability of current CMS dosage regimens to meet the above-mentioned fAUC/MIC targets but also to design optimised dosage regimens.

How Appropriate Are Current Dosage Regimens?

As previously discussed, colistin retains significant activity in vitro against many Gram-negative 'superbugs' and is often the only therapeutic option available to treat infections by these MDR Gram-negative pathogens (Bratu et al. 2005; Li et al. 2006b; Antoniadou et al. 2007; David and Gill 2008; Landman et al. 2008; Michalopoulos and Karatza 2010). With resistance to colistin beginning to emerge (Li et al. 2006b; Antoniadou et al. 2007; Ko et al. 2007; Johansen et al. 2008; Al-Sweih et al. 2011; Bogdanovich et al. 2011; Lee et al. 2011; Mezzatesta et al. 2011), it is imperative to administer CMS in regimens that maximise antibacterial activity and minimise resistance development, while also minimising the potential for adverse effects (e.g. nephrotoxicity). Unfortunately, a lack of information on the PK and PD of colistin and CMS has led to confusion regarding the 'optimal' dosing schedule (Nation and Li 2009). Current dosage regimens are primarily derived from manufacturers' package inserts that were developed decades ago, before an

understanding of modern PK/PD concepts. As discussed above (see section 'Inconsistent Labelling and Dose Regimens of Pharmaceutical Products'), the daily dosage recommendations for parenterally administered CMS differ substantially among products that are used in various regions of the world; this causes confusion and potentially leads to a situation of sub-optimal use.

In the product package inserts, the recommended number of CMS doses per day is 2–4 for a person with normal renal function, with 2–3 doses daily being a common regimen (Bergen et al. 2008). Once-daily regimens have also been employed despite a lack of supporting PK/PD data (Gunderson et al. 2003; Rosenvinge et al. 2005), presumably to take advantage of the concentration-dependent activity of colistin that is evident in vitro. However, in an in vitro PK/PD model that simulated human dosing regimens incorporating higher doses of CMS administered once daily, there was greater emergence of resistance in *P. aeruginosa* than occurred with a thrice-daily regimen involving essentially the same total daily dose (Bergen et al. 2008). Furthermore, a study conducted in rats involving week-long multiple-dose regimens mimicking once- and twice-daily administration in humans of the same daily dose of CMS, to achieve clinically relevant plasma colistin concentrations, resulted in a greater range and severity of renal lesions with the once-daily dosing regimen (Wallace et al. 2008a). In vitro studies have shown that the toxic effects of colistin on mammalian cells is both concentration- and time-dependent (Lewis and Lewis 2004). Finally, colistin lacks a significant postantibiotic effect (Li et al. 2001b; Owen et al. 2007; Poudyal et al. 2008; Ozbek and Senturk 2010). Thus, higher doses administered less frequently may potentially increase both nephrotoxicity and resistance development, although this remains to be confirmed in patients.

Evidence is emerging that the PK of CMS and formed colistin differs across various patient groups, and that currently used dosage regimens of CMS are likely to generate sub-optimal exposure to colistin in many patients. Li et al. reported a study in which 12 people with cystic fibrosis (age range 13–39 years, body weight range 39–68 kg, all with normal serum creatinine) were administered intravenously 1–2 million international units (IU) of colistin methanesulfonate every eight hours (equivalent to 1.83–3.50 mg of colistin base activity/kg per day) (Li et al. 2003a). The plasma CMS and colistin concentrations across a dosage interval at steady state are shown in Fig. 14.5. The peak plasma concentration of colistin was typically found in the first blood sample collected following the CMS dose and the half-life of the formed colistin was ~4 h. The range of calculated plasma colistin C_{max} at steady state was 1.2–3.1 mg/L while that for the minimum plasma concentration (C_{min}) was 0.14–1.3 mg/L, and the range of AUC over 24 h for formed colistin was 16–53 mg h/L (Li et al. 2003a). Even without consideration of protein binding, plasma colistin concentrations in many cases failed to reach the CLSI breakpoint of 2 mg/L (Clinical and Laboratory Standards Institute (CLSI) 2012) defining susceptibility to colistin for *P. aeruginosa* and *A. baumannii*, with plasma concentrations falling rapidly below this level even when achieved. It is apparent that the CMS dosage regimens employed and the resulting exposure to plasma colistin in these patients was very likely to be sub-optimal, especially with CMS monotherapy.
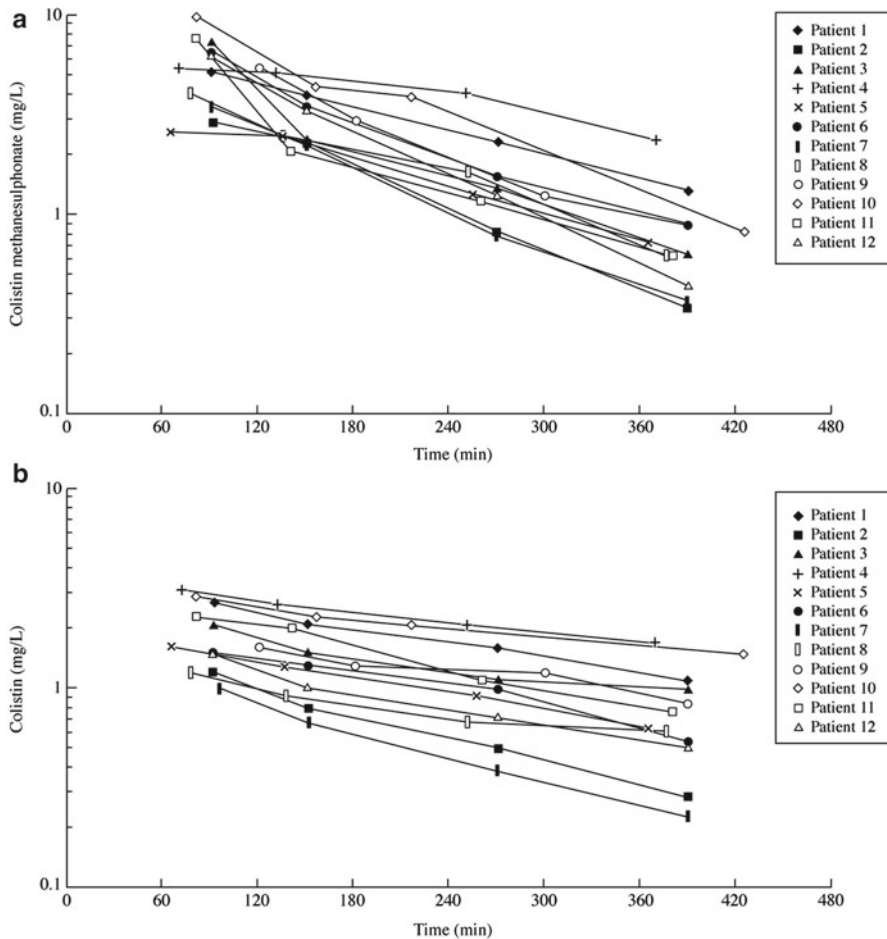
Fig. 14.5 Plasma concentrations of (**a**) colistin methanesulfonate and (**b**) colistin at steady state in 12 patients with cystic fibrosis following intravenous administration of colistin methanesulfonate 1–2 million IU every 8 h for at least 2 days. Reproduced from (Li et al. 2003a) with permission from Oxford University Press

It is increasingly obvious from studies undertaken in critically ill patients that the CMS dosage regimens used in many of these patients generate plasma colistin concentrations that are not likely to be very effective, especially when used as monotherapy (Li et al. 2005b; Markou et al. 2008; Plachouras et al. 2009; Fernandez et al. 2010; Imberti et al. 2010; Garonzik et al. 2011; Mohamed et al. 2012). Arguably, a 2005 report was the first to draw attention, based upon experimental data, to the lack of PK information for CMS and formed colistin in critically ill patients and to the lack of appropriate CMS dosage guidelines for these patients (Li et al. 2005b). Li et al. reported the disposition of CMS and formed colistin at steady state in a critically ill adult patient requiring CMS for treatment of an infection caused by MDR

P. aeruginosa. The patient had multiple organ failure requiring continuous venovenous hemodiafiltration (CVVHDF). Intravenous CMS (equivalent to 150 mg colistin base activity every 48 h) was administered as last-line therapy; the regimen was based upon the product information which suggested that in patients with renal impairment the size of the dose should be essentially maintained and the dosing interval should be increased from the normal 8–12 h. The dose actually administered to this patient was also in accord with the suggestion made, without any supporting data whatsoever, in an influential report focussing upon antibiotic dosing in critically ill patients receiving continuous renal replacement therapy (Trotman et al. 2005). Li et al. demonstrated that both CMS and colistin were cleared by CVVHDF. Importantly, total plasma concentrations of formed colistin fell below the MIC for the infecting strain ~4 h after CMS dosing. Unfortunately, 12 days after commencing CMS therapy, the patient died. Clearly, dosage adjustment for CMS in CVVHDF patients should be much more modest than that used in this patient. Subsequent studies have confirmed both CMS and colistin are efficiently cleared by intermittent hemodialysis and continuous renal replacement therapy (either CVVHDF or continuous venovenous hemofiltration) (Marchand et al. 2010a; Garonzik et al. 2011).

Makou et al. reported plasma colistin concentrations across a CMS dosage interval at least 2 days after commencing therapy (Markou et al. 2008); all patients, who were adults, had creatinine clearance >46 mL/min and 13 of the 14 patients received 2.8 million IU CMS intravenously 8 or 12 hourly (corresponding to ~270 mg colistin base activity per day). The range of C_{max} values for formed colistin was 1.15–5.14 mg/L while that for C_{min} was 0.35–1.70 mg/L; the AUC over 24 h for formed colistin ranged from 12.8 to 60.0 mg h/L. The authors expressed concern about the low plasma concentrations of colistin achieved in these patients. It was not surprising, given that all patients had moderate to good renal function, that Makou et al. were not able to discern a relationship between plasma colistin C_{max} or C_{min} and creatinine clearance. Imberti et al. reported plasma colistin concentrations across a dosage interval at least 2 days after commencing therapy in 13 adult critically ill patients with ventilator-associated pneumonia caused by Gram-negative bacteria (Imberti et al. 2010). Each patient had a creatinine clearance of >96 mL/min and received CMS two million IU intravenously 8 hourly (equivalent to ~180 mg colistin base activity per day). There was no apparent relationship between plasma colistin C_{max} (range 0.68–4.65 mg/L), C_{min} (0.23–2.43 mg/L) or AUC over 24 h (8.9–75.2 mg h/L) and creatinine clearance; as with the study of Markou et al. (2008), failure to identify a relationship between colistin PK parameters and renal function is not at all surprising given that all patients had creatinine clearance values around 100 mL/min or greater. In the study of Imberti et al. bronchoalveolar lavage (BAL) was performed 2 h after administration of a CMS dose (Imberti et al. 2010). The authors did not concentrate the BAL fluid prior to analysis to increase the sensitivity of the assay, and the colistin concentration was below the limit of detection (0.05 mg/L). It is not possible to interpret this finding because of the extensive dilution of pulmonary epithelial lining fluid (ELF) that occurs during the BAL procedure. For example, if ~100-fold dilution occurs then even if the actual concentration of colistin in BAL fluid was 0.04 mg/L this would be equivalent to 4 mg/L in ELF.

Two studies by the same research group have made a useful contribution to the understanding of important facets of the disposition of CMS and formed colistin in adult critically ill patients (Plachouras et al. 2009; Mohamed et al. 2012). In the first study, the plasma colistin concentration–time profiles observed with an intravenous CMS regimen of 3 million IU 8 hourly (equivalent to ~270 mg colistin base activity per day) in 18 critically ill patients (creatinine clearance range of 41–126 mL/min), without administration of a loading dose, revealed that total plasma colistin concentrations remained well below the MIC breakpoints for the first few doses in the regimen (Plachouras et al. 2009). Indeed, the predicted plasma colistin C_{max} from that study was 0.60 mg/L after the first dose, while plasma colistin concentrations ≥2 mg/L were not achieved until approximately 44 h after commencing therapy; the typical plasma colistin C_{max} at steady state was estimated to be 2.3 mg/L. Even at steady state, the plasma concentrations of formed colistin were below the MIC breakpoint in many patients, without consideration of plasma protein binding. In a follow-up study, the same group reported clinical PK data on a further ten critically ill patients (creatinine clearance range of 24.9–214.3 mL/min; intravenous CMS maintenance doses of 1–3 million IU 8 hourly) (Mohamed et al. 2012). The PK data were analysed simultaneously with the data from the original study (Plachouras et al. 2009). Once again, steady-state plasma colistin concentrations were not achieved for 2–3 days, were low (the average colistin C_{max} at steady-state was 2.3 mg/L) and a large fraction of the patients had plasma colistin concentrations below the MIC breakpoint of 2 mg/L (Mohamed et al. 2012). Importantly, delayed initiation of appropriate antimicrobial therapy is associated with increased mortality in critically ill patients (Kumar et al. 2006; Luna et al. 2006), and low colistin concentrations have been associated with the amplification of colistin-resistant subpopulations (Tan et al. 2007; Bergen et al. 2008, 2011a, b; Poudyal et al. 2008; Bulitta et al. 2010; Dudhani et al. 2010b). Mathematical modelling by Bulitta et al. predicted colistin regimens with a large colistin exposure during the first ~12 h may be beneficial, providing enough net killing such that the immune system may be able to eradicate any remaining colistin-resistant cells (Bulitta et al. 2010). Thus, it is evident from the data presented by Plachouras et al. and Mohamed et al. that therapy with CMS should commence with a loading dose, which was suggested by the authors (Plachouras et al. 2009; Mohamed et al. 2012). Because of the potential for nephrotoxicity, it is suggested that the loading dose for an adult should not exceed 300 mg colistin base activity, with the first maintenance dose administered 24 h later (Garonzik et al. 2011). Because there were only 28 patients in total in the two studies reported by Plachouras et al. and Mohamed et al., with all but one patient having a creatinine clearance of >41 mL/min, it was not possible for these investigators to identify any relationships between the CMS or colistin kinetics and renal function (Plachouras et al. 2009; Mohamed et al. 2012).

The impact of renal function in critically ill patients on the disposition of CMS and formed colistin is evident from the largest pharmacokinetic study to date involving 105 patients, 89 of whom had very diverse renal function (creatinine clearance 3–169 mL/min/1.73 m^2) but were not receiving renal support, 12 who were receiving intermittent hemodialysis and 4 who were recipients of continuous renal
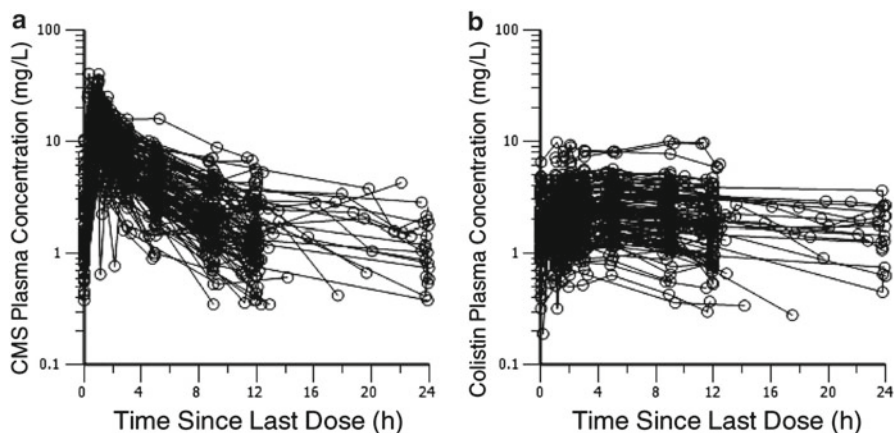
Fig. 14.6 Steady-state plasma concentration-time profiles of (**a**) colistin methanesulfonate and (**b**) formed colistin in 105 critically ill patients (89 not on renal replacement, 12 on intermittent hemodialysis and 4 on continuous renal replacement therapy). The physician-selected daily dose ranged from 75 to 410 mg colistin base activity; the dosage intervals ranged from 8 to 24 h and hence the inter-dosing blood sampling interval spanned the same range. Reproduced from (Garonzik et al. 2011) with permission from the American Society for Microbiology

replacement therapy (Garonzik et al. 2011). The plasma concentration-time profiles of CMS and formed colistin across a dosage interval at steady state in the 105 patients who were not receiving renal support are presented in Fig. 14.6. It is evident that within each patient there was generally little fluctuation in the plasma colistin concentrations across a dosage interval, consistent with a protracted half-life for formed colistin in these very sick patients. The CMS dosage regimens administered to these patients (median daily dose across the 105 patients was 200 mg colistin base activity; range 75–410 mg colistin base activity per day) achieved average steady-state plasma colistin concentrations of 0.48–9.38 mg/L (median, 2.36 mg/L; Fig. 14.6), corresponding to a range of AUC over 24 h of 11.5–225 mg h/L. That is, the ~5.5-fold range of CMS daily doses generated a ~20-fold range of exposure to colistin in plasma. The importance of renal function as a determinant of the plasma colistin concentrations achieved from a given daily dose of CMS can begin to be appreciated from the data presented in Fig. 14.7. Indeed, population PK modelling revealed that creatinine clearance was an important covariate for the clearance of CMS and the apparent clearance of colistin. That the clearance of CMS correlated with renal function is not surprising given that CMS is predominantly cleared by renal excretion. However, the fact that creatinine clearance was a covariate for the apparent clearance of formed colistin may, at first thought, seem rather surprising because colistin is mainly excreted by non-renal mechanisms (see section 'Pharmacokinetics, Pharmacodynamics and Pharmacokinetic/Pharmacodynamic Relationships'). The explanation lies in the schema shown in Fig. 14.2. In patients with relatively normal renal function, only a very small fraction of an administered dose of CMS is converted to colistin because the renal clearance of CMS is
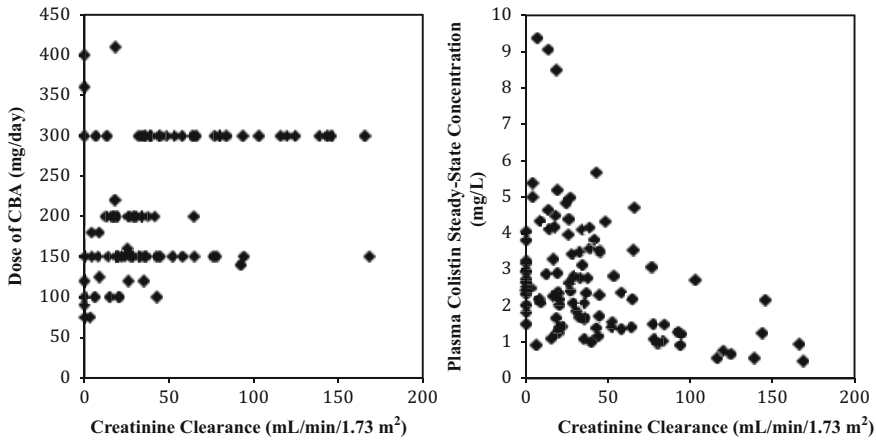
Fig. 14.7 Relationship of (**a**) physician-selected daily dose of colistin base activity (CBA) and (**b**) the resultant average steady-state plasma colistin concentration with creatinine clearance in 105 critically ill patients. Reproduced from (Garonzik et al. 2011) with permission from the American Society for Microbiology

substantially greater than the clearance for the formation of colistin from CMS. However, in patients with substantial reductions in kidney function, the renal clearance of CMS declines and consequently a greater fraction of the administered dose of CMS is converted to colistin. Thus, the apparent clearance of colistin correlates with creatinine clearance, leading to at least two important practical consequences. First, it is evident that in patients with moderate to good renal function, administration of a daily dose of colistin base activity at the upper limit of the current product-recommended dose range (300 mg colistin base activity per day) (Coly-Mycin 2005) was not able to generate plasma colistin concentrations that would be expected to be reliably efficacious (Fig. 14.6). The second practical consequence is that reduction of the daily dose of CMS is required as renal function declines, in patients who are not receiving renal support with either intermittent hemodialysis or continuous renal replacement therapy. In agreement with previous case reports concerning critically ill patients on intermittent hemodialysis Marchand et al. (2010a) or continuous renal replacement therapy (Li et al. 2005b), both CMS and colistin were shown to have relatively efficient extracorporeal clearance in the 12 and 4 patients, respectively, who were receiving these forms of renal support (Garonzik et al. 2011). A very important practical outcome of this study was the generation of the first scientifically based CMS-dosing algorithms for patients with a large range of renal function, including patients on intermittent hemodialysis or continuous renal replacement therapy; the algorithms allowed calculation of the CMS loading and maintenance doses required to achieve a desired target average steady-state plasma concentration for colistin. Overall, the observations from this study (Fig. 14.7) are a cause for significant concern, suggesting suboptimal exposure to formed colistin with current CMS dosage regimens, particularly when one or more

of the following applies: (1) the patient has moderate to good renal function, in which case it is unlikely that even a CMS daily dose at the upper limit of the product-recommended dose range will generate plasma colistin exposure likely to be reliably efficacious; (2) MIC of the infecting strain is in the upper range (i.e. 2 mg/L) of the susceptibility region for colistin; and (3) the infection is associated with high bacterial numbers. Under these circumstances, the most appropriate approach is likely to be therapy with a rationally selected colistin combination regimen.

Conclusions

The last several years have seen significant advances in unravelling of key aspects of the pharmacokinetics and pharmacodynamics of colistin and the relationship between the pharmacokinetics and pharmacodynamics, resulting for the first time in the generation of scientifically based dosing algorithms for CMS. As both preclinical and clinical investigations continue there will be further steps towards understanding how to optimise the administration of colistin methanesulfonate. The future incorporation of human PK/PD data into dosing algorithms, including information on colistin plasma protein binding and endpoints such as clinical cure, bacteriological eradication and the development of resistance will be very important. In addition, studies examining colistin monotherapy versus combination therapy will facilitate further optimization of colistin therapy in the various categories of patients who now require this important last-line antibiotic against Gram-negative pathogens.

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